

Schools for another century

The British government is rightly seeking to improve the quality of school education. It must first win back the confidence of teachers and then act generously.

How should young people be prepared for life in the brave new world thought likely to begin in 2000 or thereabouts? That question preoccupies educationists in industrialized countries as different as the United States and Japan. Most of the recipes emerging from this introspection entail the assumption that the next century will make greater demands on the numeracy of the general population, which is a fair guess. Governments in the industrialized countries are also conscious that their survival in recent years has hung on economic competition in the design and manufacture of the products of technological innovation, whence the general objective that educational systems must produce many more young people qualified to engage in this competition. That, too, is a reasonable proposition. The difficulty, as all reformers have discovered, is that it is much easier to specify what the future will require of schools and universities than to arrange matters so that these consequences obtain.

This is the test that must be applied to the torrent of proposals for educational reform now flowing from the British government's Department of Education and Science. Last week, Sir Keith Joseph, the minister in charge, published a policy statement called *Better Schools* whose proposals would transform the way in which schools are organized. This has been quickly followed by a set of detailed proposals for a new public examination to be taken by all British sixteen-year-olds that will break new ground in at least two important ways — the General Certificate of Secondary Education is meant to be a measure of educational attainment for students whose ability spans the whole spectrum, while the grades awarded under the new system will be determined by standards supposed to be objective (and not, as with existing school-level examinations, by competition for a fixed quota of the higher grades). For good measure, the government has also published a policy statement on science education urging that all students should follow science courses from the ages of 5 to 16. Objectively, the new proposals are both enlightened and courageous. But ironically, they have appeared at a time when the British government is in dispute with school teachers over pay. So Sir Keith's rush at reform has won him more enemies than friends. He should not be too surprised.

Improvements

Part of the difficulty is that the minister, like the government of which he is a member, has plainly been forced by the realities of the educational system to abandon some of the prejudices with which it came to office in 1979. British schools were then still adjusting in the wake of the upheavals of earlier decades — the reorganization of secondary schools to provide a comprehensive education within their catchment areas, and the wave of curriculum development that enlivened much teaching but which also threw a disconcerting light on the poverty of the rest. In retrospect, the comprehensive reorganization, socially and even administratively a necessity, was forced through too quickly, with the result that the performance of both teachers and students fell below par. The present government's first instinct was to unscramble the reorganization, but by 1979, the omlette was indissoluble. So attention turned to the improvement of educational standards within the publicly supported schools. That is the starting point for *Better Schools*.

There are some things to boast about. The proportions of

students leaving secondary schools with qualifications in public examinations have risen over the past two decades, if slowly. The government is able to claim that three-quarters of school-leavers now have graded examination results in at least five subjects. The other side of that coin is that only just over a quarter of them perform creditably, well enough to be able to use their qualifications in persuading employers to offer them jobs. Some of the reasons are spelled out in *Better Schools*. In secondary schools, the planning of the curriculum is too often left to chance. Language-learning is almost universally undervalued. Too many teachers are required to perform beyond the levels for which their training and capacity have equipped them. And so on.

Changes

Complaints such as these have been familiar in British education, as in educational systems elsewhere, for decades. The underlying difficulty centres on the sociology of the school. As in other kinds of educational institutions (universities, for example — see below) the polite conventions of academic life conspire to allow teachers the freedom to teach badly if that is their inclination. In the long run, the only remedy is that schools should become more coherent institutions, places in which professional teachers are involved both with the determination of a school's objectives and with the planning of how flesh should be put on these bones. Too often, in the past, bureaucratic interference from outside, or the waywardness of those in charge of schools, has had the effect of giving teachers the impression that they are merely hired functionaries. One of Sir Keith Joseph's more courageous ambitions is to change all this. Among other things, he plans that professional training courses should be more thorough, that the performance of teachers in posts should be regularly assessed, and that teachers' pay should be linked to performance. Nobody disagrees with these objectives. Why have they landed the minister in hot water?

The most obvious difficulty is that the talk of teacher assessment which has been buzzing about for more than a year has not yet been embodied in a set of tangible proposals that teachers trust. Who will carry out the assessment, and by what criteria? Teachers may be forgiven for asking that these questions should be answered before they entrust their careers to such an innovation, however desirable its effects may be. Yet even now, in last week's flurry of documents on educational policy, it is clear that the government has not made up its mind. There is talk of assessment by local education authorities (which employ teachers) and of the possibility that there may have to be some national scheme backed up by a statutory definition of a teacher's responsibility. But the only level at which the assessment of a teacher's performance can be undertaken sensitively is that of the school. The British government would find its ambitions more easily attainable if schools were given a greater degree of autonomy than they enjoy at present, for then professional teachers would more easily sense the connection there must be between performance, success and security.

Another source of difficulty is that the British government's financial support for schools has become ungenerous. As with British universities, British schools are now kept on tight budgets. The government can claim (as it does in *Better Schools*) that school budgets have not declined as quickly as have school



enrolments, but there are still far too many schools short of resources that they must look to parents for essential provisions of cash. And teachers, unassessed though they may be, are badly paid on rigid salary scales allowing for very little variation in the way in which outstanding members of the profession may be rewarded. The government now seems to be saying that it will devise more imaginative ways of rewarding teachers only when the profession has accepted the need for assessment (and has also swallowed this year's offer on pay). That is too wooden a posture. The government would be better advised to take a more active role in persuading teachers to its convictions. One of the essential ingredients of this process should be an undertaking that the resources spent on school education will be substantially increased as the years go by.

For that, in the long run, is the only way in which the government's ambitions to improve educational standards can be attained. The objective should be that the quality of the publicly supported schools should be comparable with that of the private schools, whose performance is a galling reminder to the public sector of how much improvement there is to make. By comparison, reform of the examinations system cannot contribute as much as would a few tangible demonstrations that it is possible radically to improve the quality of education with Britain's impoverished public sector. For Sir Keith Joseph, the implications should be plain. He cannot hope to bring about his reforms without the willing collaboration of a teaching force whose confidence he has lost. The remedy, painful though it may be, is that he must win them round, chiefly by talk but partly by means of some simple demonstrations of the improvements he rightly wants to see. He may have thought his rash of policy documents would tidy up this important enterprise, but it is only a beginning. □

University business

British universities should seize an opportunity for reform.

THE report of the Jarrett committee on the efficiency of British universities has turned out not to be the fearsome document that some academics had expected. But that does not imply that its modest and sensible proposals will be adopted without difficulty or, for that matter, ignored. The essence of the argument now advanced (see p.394) is that, in spite of the distinctiveness of their social function, there is no reason why universities should not be managed as are other complicated institutions, by the self-conscious planning of their own development and by repeated re-examination of the match (or mismatch) between their resources and their ambitions. On that point, the Jarrett committee is on entirely sure ground. The practical difficulty, during the past four years but probably for some time to come, is that individual universities must draw up strategic plans in ignorance of the resources they will have at their disposal in the future. Crisis management has been the game since the budget cuts of 1981. Planning for uncertainty is now the order of the day.

Come what may, British universities cannot emerge qualitatively unchanged from this period of upheaval, for which Jarrett is merely the latest stimulus. The issue that confronts them is simple, even stark. Faced with shrinking resources, the University Grants Committee, British universities' paymaster, has been compelled to plan to distribute what funds there are in a discriminatory fashion. Something like two-thirds of its pot of gold will be used for the support of teaching, the rest for providing the infrastructure for research. The first task may in due course be done by means of formulae, but the second will entail some necessarily invidious judgements on whether this or that research is academically more interesting, or otherwise more deserving. People (*Nature* included) will be watching the grants committee like hawks to ensure that it does not sacrifice quality in the face of claims (from the government, perhaps) that second-rate work may be more useful, but universities cannot hope to succeed in this environment if they are not equipped to be internally discriminatory (between the good and the less good). That, briefly,

is all that Jarrett recommends.

The objections are easily anticipated. Universities everywhere, jealous though they are of their constitutional autonomy, have traditionally shrunk from suggestions that they should apply yardsticks to the quality of the work their people do. It is a painful process, but also one in danger of being corrupted by the special influence of powerful members of a faculty, more able to fight for their own corners than they deserve to be. There will also be cries that attempts to discriminate between different fields of research by the allocation of resources will be an offence against academic freedom, which is a beguiling half-truth. For academic freedom implies merely that an academic should be free to pursue the goals he judges to be worthwhile, not that he should be equipped to do so on some standard scale. Yet in practice, the difficulties of deciding these contentious questions within an exclusively academic body are so great that academics should themselves recognize that it will be simplest if the responsibility can be shared with the university's governing body. That is the logic of the Jarrett recommendation. Nobody should think that it will be easily effected.

Much the same is true of the proposal that members of academic staffs at British universities should be regularly subjected to formal appraisal of performance as are, for example, people who work in business or in the civil service. The Jarrett report remarks that appraisal of a person's performance in academic life now happens only accidentally, perhaps at the end of a probationary period or at promotion or relocation. (It might have added that the criteria used on these occasions are often too narrow, or too unambitious.) Again there will be fears that a formal requirement that senior academics should tell their junior colleagues how they rate will interfere with academic freedom, but this is also mistaken. Jarrett might have made more of the positive benefits of sensitive assessment. As things are, too many British universities wedded to the notion that young academics will either sink or swim are allowing their indifference to the careers of new recruits to become a means of wasting talent.

Most British universities will no doubt read the writing on the wall, and bite the bullets Jarrett offers. What, however, will happen to those that are constitutionally incapable of responding? Oxford and Cambridge, which are not merely autonomous but which are self-governing to an embarrassing degree, may find themselves having to vote away some of their high-level mob rule. The University of London, by any standards the largest in the United Kingdom, will be in greater difficulties. With its federal structure, the obvious danger is that the central administration will blunt the efforts of its constituent parts to improve their capacity to make decisions. The simplest solution is to allow these parts, all of them now larger than many other British universities, to deal directly with the grants committee. Jarrett's management principles require no less.

Whether reforms along these lines will serve the intended purpose does not, unfortunately, rest with the universities, or even with the grants committee. Increasingly, it is the government that determines policy, but on a short-term basis. The first round of budget cuts decreed in 1980 was based on the doctrine that higher education must bear its share of the general reduction of public expenditure then planned; in the event, it cost the government an unplanned £80 million to send 5,000 academics into early retirement. Now there are budget numbers for the three years ahead, but nobody has explained how the university system is to manage the further contraction they imply. And the government is still silent on the issue of how many students should be admitted to British universities, for what purposes and with what support. The general exhortation that universities and industry should work more closely together is known to have financial implications still undefined. All these questions were supposed by now to have been clarified in a policy paper from the British government, whose publication has been imminent for nearly a year. Sir Keith Joseph, the minister responsible, may have had other things on his mind (see above), but his apparently endless delay in saying what he wants of universities is also bad management of a kind. □

US universities

Now even the Texans are short of cash

Austin, Texas

TEXAN ambition is legendary, and the state's two major campuses, the University of Texas (UT) at Austin and Texas A & M University at College Station, certainly fit into that mould. Both aspire to be counted among the country's top research institutions before the end of the century, and both can point to impressive steps already taken towards that goal, including the well-publicised poaching of academic superstars from better-known and more widely celebrated institutions. But a state budget crisis is casting a shadow over the universities' finances — both are state supported — and faculty recruitment and teaching programmes may suffer as a result.

The Texan economy has been built on oil. Since oil prices started to fall two years ago there have been growing numbers of business failures, and property prices have fallen, sometimes dramatically. State tax revenues have also fallen, and because the state legislature is constitutionally prevented from running a budget deficit it now has to find ways of reducing spending by almost \$1,000 million (the alternative, raising tax rates, seems too unpopular even to contemplate in Texas). The first proposal for achieving this saving, drafted against a deadline and hardly intended to be taken seriously, would have done so entirely by reducing the state's higher education budget by a startling 29 per cent. The most recent proposals are more moderate — it seems to be agreed that the 1986 higher education appropriation should not be less than 96 per cent of that for 1985 — but the state House of Representatives appropriations committee has nevertheless approved a plan to close down the UT Permian Basin campus and the A & M campus at Galveston. While this will not be the last word on the plan, it indicates how seriously the crisis is now being taken.

Many fear that even small cutbacks will damage the Texas "can do" reputation, which is held to be the major reason why both universities have been so successful in building first class academic departments. Eight UT Austin PhD programmes were ranked among the top ten in the country by a recent national survey, and A & M claims to be among the top 20 US universities in research expenditure. The chancellor of the UT system is on record as saying that support at 96 per cent of 1985 levels will be insufficient to keep academic programmes at their current levels, however, and the university is still pressing for a 4.9 per cent increase. The funding decisions by the legislature are supposed by law to be decided by midnight of 31 May;

this year many expect the legislature to resort to the device (used on some previous occasions) of literally turning back the chamber's clock to gain extra time.

The state budget crunch is doubly unfortunate because it comes at the same time as legislative changes that will reduce the benefits accruing to UT Austin and Texas A & M from a massive land endowment granted by the state last century. The 2 million acres of west Texas that were set aside to support scholarship were later found to lie above substantial oil deposits, and it is principally the oil that has supported the extraordinary expansion of both institutions since the 1920s. Royalties are channelled into a Permanent University Fund, now worth around \$2,000 million; interest from the permanent fund constitutes the Available University Fund, amounting to about \$150 million a year. The universities use the permanent fund to guarantee loans for new building construction, and interest on this bonded indebtedness is the first call made on the available fund.

In a ballot last November, the state increased the number of campuses eligible to use the Permanent University Fund to guarantee loans, to include virtually all UT and A & M campuses; this privilege had previously extended only to a few sites, principally the large College Station and Austin campuses. The result will be that more of the available fund will be tied up servicing debts and so proportionately less will be available for other improvements at the main campuses.

Despite the squeeze, both universities have more to be cheerful about than most: UT's total endowments are by some reckonings worth more even than those of Harvard, generally thought to be the wealthiest university in the country. And the president of Texas A & M, Frank Vandiver, is adamant that state budget problems will not impede plans to recruit top quality faculty: top salaries can be paid to attract the right people (there are two faculty members earning over \$100,000 a year) can be paid to attract the right people, and, even more important, the university will provide buildings and equipment beyond the means of many institutions. The success of A & M in becoming the scientific headquarters of the Ocean Drilling Program is ascribed largely to Vandiver's personal commitment to provide \$5 million of university funds for a new building for the programme, the first on the university's research park. A & M is also making a strong pitch to attract the proposed Superconducting Supercollider to Texas, to which end it has already devoted \$1 million.

Perils of secrecy

Washington

THE Office of Technology Assessment (OTA), which exists to advise Congress on technical matters, has discovered one of the pitfalls of working with classified documents. The office was recently commissioned by a congressional committee to prepare a report on "C3I" — command, control, communications and intelligence of nuclear forces. OTA's researcher was provided with the appropriate security clearances; the final report was to be classified.

After poring over Department of Defense (DoD) documents, OTA's researcher wrote his report and submitted it to DoD for security review. DoD promptly slapped on it a classification even higher than the level for which the researcher had had clearance. The classification is also higher than congressmen are ever allowed. So now neither OTA nor the committee that commissioned the study is allowed to read it.

The explanation is that the OTA researcher apparently pulled together enough top secret and "compartmentalized" data (one step above top secret — those with access to any given compartment are not given access to any others or even permitted to know what other compartments exist) to reproduce some of DoD's most secret data on nuclear war-fighting plans, which have a security classification all their own.

OTA officials have suggested outfitting their researcher with a blindfold to allow him to remove the offending passages.

Stephen Budiansky

At UT Austin, recruitment is now under way to fill 32 new scientific professorial chairs, each carrying an endowment of \$1 million. Last year, the university accepted \$16 million in private donations for the chairs and was able to match this sum from the Available University Fund; the first appointments have now been announced. The 32 chairs will be in natural sciences and engineering, with a strong emphasis on molecular biology and materials sciences, reflecting the university's desire to serve the economic needs of society, according to Austin Gleeson, dean of the college of natural sciences. Some faculties outside the favoured areas are resentful of what they see as a lack of consultation by the university on where the money should be spent. But Gerhard Fonken, Dean of academic affairs, argues that when a single gift of \$8 million is being discussed it is well to bear the donor's wishes in mind. The first of the 32 appointments have recently been announced, but already there are worrying signs that some scientists who have been approached are being deterred by reports about the state legislature's early proposals for higher education spending. The view at Austin is still firmly optimistic; talk of faculty lay-offs is dismissed as scaremongering.

Tim Beardsley

British universities

Better management demanded

BRITISH universities are in for another shake-up, this time a revolution engendered from within. So much is clear from the report published earlier this week of the committee under Sir Alex Jarrett set up a year ago to give the Committee of Vice-Chancellors and Principals advice on the effective management of universities.

The study now completed has been an unusual exercise, in which individual academics collaborated with professional management consultants and firms of auditors to look into the efficiency of six institutions (the universities of Edinburgh, Essex, Loughborough, Nottingham, Sheffield and University College, London). The report now published is that of the steering committee for the separate studies, and seeks to emphasize some general conclusions reached.

The report seems to accept that the financial deprivation of the British university system will continue, and perhaps even intensify. The committee notes that the hope that the severe cuts of 1981 (effectively a reduction of budgets by 17 per cent over three years) have not been followed by the once-promised "level funding" but by a period during which central government support for universities has been fixed in cash terms that do not adequately compensate for inflation.

The committee notes that, during this period, the British government has also taken steps to influence the pattern of university development, both by the encouragements of some kinds of academic developments and by limitations of student numbers. The report pleads that, in the interests of efficiency, the government should make clear its intentions towards the British university system and that it should look for some way of enabling universities to plan more than one year ahead. Specifically, the committee asks that the government should be prepared to meet the "whole or the greater part" of the cost of such academic redundancy as may be needed in the future.

Starting from the view that change within universities will be further stimulated by the determination of the University Grants Committee to distribute funds more selectively in future, the committee argues for more decisive management within universities. It says that governing bodies (variously called "councils" and "courts" in the British system) should assume responsibility for strategic planning and that vice-chancellors (also called principals) should be recognized as the chief executives as well as academic leaders of institutions (and be given increased powers to do so when necessary).

Ordinarily, the Jarrett report says, there should be a joint committee of the council and the academic senate, with the vice-chancellor as chairman, to superintend

planning and the allocation of resources within a university. At the department level, the report says, steps should be taken to see that the performance of individual academics, as teachers and in research, is regularly assessed. In general, the report asks that universities should prune the numbers of committees dealing with other than academic matters, delegating affairs such as the operation of student residences

Japanese whaling

Hollow victory for United States?

Tokyo

UNSUSPECTING foreigners out in Roppongi, the nightlife centre of Tokyo, are in for a surprise, for there amongst the flashing neon is a large sign in English, paid for by the All Japan Seaman's Union, reading "Continue the whaling on scientific facts". These words, designed to strike at the heart of logically minded Westerners, may be the last from a dying industry.

Scientific facts or no scientific facts, decisions are rapidly being made about the future of whaling in Japan and, despite last-ditch diplomatic efforts by Japan's Fishery Agency, concessions are having to be made that almost certainly spell doom for the offshore whaling industry.

In December last year, the Japanese government filed a formal objection to the 1984-85 International Whaling Commission (IWC) quota for the minke whale catch in the Antarctic Ocean — minke whaling forming the backbone of the Japanese whaling industry. The IWC quota had been set at 4,224 head, 37 per cent down from the previous year, of which 1,941 head were allocated to Japan. By filing the objection, Japan freed itself from the need to observe any quota at all; soon after, however, Japan announced it would "voluntarily" limit Antarctic minke whaling in the November 1984/March 1985 whaling season to the previous season's levels (3,027 head). This, however, did not appease world opinion sufficiently for the United States to withdraw its threat to invoke the Packwood-Magnusson Amendment, a domestic ruling to cut the fishery catch in US waters of any country that defied IWC quotas. This would mean that Japan's huge fishing quota in the US 200-mile fishing zone would be halved in the first year of defiance and reduced to nothing in the second. To buy time for talks, a further compromise was made: the Japanese Antarctic whaling fleet was ordered to stop catching on reaching the new quota set by IWC and return home.

But the move did little to ease US pressure, and a series of talks between the United States and Japan has led to an ultimatum that Japan must withdraw an objection it filed earlier to the IWC complete ban

and the maintenance of buildings to university officials.

The report also asks that the grants committee should itself be more active and articulate, and that it should be more vigilant in monitoring promises made by individual universities (as when, in 1981, universities agreed to close some departments and to run others in collaboration with neighbouring institutions). The Jarrett report also says there should be a review of the grants committee itself, in part so as to tell whether it is sufficiently well-provided with resources for the work it has to do. □

on commercial whaling that is to begin in 1986. Withdrawal of the objection would effectively mean complete defeat: all Japanese commercial whaling would have to end by 1988. All that the Japanese have so far won from the United States is an extra two years' grace — 1988 rather than 1986 — before they must observe the moratorium. Even this gesture was hotly contested in the courts by US environmentalists. A last-minute attempt to gain further concessions from the United States is under way this week, but the Japanese government has already admitted that little can be expected.

The implication of the Seaman's Union poster is that there is actually no clear scientific reason to stop minke whaling. Even IWC's own scientific committee had not claimed that minke stocks were in danger of extinction. Not surprisingly then, the Japanese see their defeat over whaling as a victory for US environmentalists and a view of the morality of the killing of whales they do not share. Strong emotions have been aroused about the way in which foreign pressure has crushed an ancient industry — indeed the Seaman's Union poster carries a different message in Japanese: "Defend our traditional whaling", and has a picture of a traditional craft with rowers clad in loin-cloths to drive home the point.

US environmentalists have recently tried to ease tension by rewarding cases of Japanese kindness to whales. A baby whale that became entangled in a shore net and ended up still alive at a fish market was recently bought by a group of fifteen Odawara fishermen and returned to the sea. Earth Trust, a US environmental group, then gave them a 20,000 yen reward together with a commendation and a whale sculpture. The fishermen, who had been moved to free the whale because "it looked as though it was shedding tears", will donate the money to African refugees.

The whales may be a lot better off for these developments but the United States may well feel a backlash. The Japanese feel they have been forced to concede and the next issue may be that much harder fought.

David D. Swinbanks

Deep-sea drilling

No cut-price membership

Washington

NATIONS participating in the Ocean Drilling Program (ODP) have come down hard against a suggestion from Britain that it might join the project as a part member, perhaps in collaboration with another country, and so save part of the £2.5 million annual membership fee. The executive committee of Joint Oceanographic Institutions Deep Earth Sampling (JOIDES), which manages ODP, has, while urging the United Kingdom to join, passed a resolution that it would be "neither appropriate nor in the best interests of the program or other full members" for Britain to join except as a full member. JOIDES has also instructed planning committees not to guarantee places for British scientists on future research missions of the new ODP drilling ship, the *JOIDES Resolution*.

Britain became a *de facto* non-member of ODP at the end of January when the British Natural Environment Research Council (NERC) failed to raise sufficient funds in time for a deadline. Britain's representative, John Bowman of NERC, had earlier voted in support of the rule that countries that had not paid up by the end of January would no longer be considered members. Efforts are still under way in Britain, however, to secure the amount needed, and JOIDES officials point out that Britain would be able to rejoin in future years if present efforts fail.

The British unwillingness to find the membership fee has annoyed other full

members, because the ODP budget had been planned on the assumption that there would be four full members (besides the United States) during 1985. Other full members are France, West Germany and Canada. Japan is committed to join after September this year, but this was also anticipated in budget planning. The programme will be \$2.5 million short next year unless another member is found. Already, purchase of some shore-based equipment has had to be deferred, according to Philip Rabinowitz, director of ODP.

ODP scientists say the British scientific contribution to ODP will also be missed. Britain, for its part, would lose its influence on the committees that plan the cruises of *JOIDES Resolution*. This is likely to cause problems for the independent British Antarctic Survey, which has planned its own work on the assumption that Britain would be a member of ODP and so able to participate in an Antarctic drilling cruise in the Weddell Sea.

Despite JOIDES' firm opposition to part membership for Britain, there are hopes that a consortium of European Science Foundation members may join in partnership with Australia. Britain, still out in the cold, has sought temporary observer membership status like that now enjoyed by Japan, and it seems likely that JOIDES will approve this request. But JOIDES is adamant that observer memberships will not be allowed to continue beyond the end of September. **Tim Beardsley**

Alternative energy

Fuels from the farm

Venice

RESEARCH in the European Community to produce ethanol fuels from biomass is to be stepped up; this may help to solve the problem of agricultural surpluses and the need for a new fuel blend policy as lead is phased out of fuel, and exhaust emissions are further reduced in the European Community (see *Nature* 28 March, p.304).

The problem of surpluses has injected new urgency in the eight-year-old research proposal, which forms part of a broad high-level Commission strategy by farm commissioner Frans Andriessen. According to Dr Strub, general director of the Commission's solar energy division, now there is the political will the research will form an important part of the Community's biomass policy, which has been allocated research funds of 20 million ECU (European Currency Units) for a four-year period.

While farm ministers last week battled over price reductions to stem agricultural overproduction, particularly in cereals, there were signs of a consensus between research, industry and the farm lobby at a European Commission-sponsored meeting on biomass in Venice. According to Madron Seligmann, a British Member of the European Parliament, one hectare of land can produce four tonnes of ethanol and one tonne of biomass each year. The nine million hectares (of the Community's 150 million hectares of agricultural land) that grow surpluses could produce 32 million tonnes of ethanol every year. The 8 million tonnes of cereal surpluses alone represents 2 million tonnes of ethanol or 1.6 million tonnes of butanol-acetone.

The cost of getting rid of Community surpluses is estimated at 7,000 million ECU. According to Professor Andre Giraud (former French industry and energy minister and initiator of the French biomass and nuclear programmes), the Community would actually spend less by encouraging biomass production from surpluses than by subsidizing them, which could be done by a gradually declining system of incentives, and closer cooperation between agriculture and industry.

The need for a new European fuel blend policy has become more pressing as Europe prepares for lead-free petrol distribution in 1989 and stricter vehicle emission controls from 1988 as a result of the decisions by environment ministers on 20 March. Oxygenated products such as ethanol, methanol, acetone and butanol are already used to enhance the octane rating of some 60 per cent of fuel sold in West Germany, which conforms to the stricter Community standard of lead content of 0.15 grams per litre. These fuels may be suitable for the lean-burn engines due shortly. **Anna Lubinska**

The sweet smell of success

Tokyo

BIOTECHNOLOGY may not yet be making much of a return for US venture capitalists, but in Japan it is already selling cosmetics just as fast as they can be put on the shelves. Recently launched and already a huge success is cosmetic manufacturers Kanebo's "Bio series" of lipsticks, eye shadow, powder and so on.

What puts the "bio" in the series is that the pigment shiokinin used in the cosmetics, is obtained by large-scale plant cell culture. Until now shiokinin has been extracted from the naturally grown roots of the plant gromwell. But in the new process, gromwell root cells are cultured in 750-litre bioreactors and the pigment recovered from solution (see *Nature* 307, 504; 1984). Interestingly, the scientists who developed plant cell culture at Mitsui Petrochemical Industries had not originally been aiming at the cosmetics market at all. Only when they found that they had developed a working system were they forced to try to find a good product to make with it — cosmetics was one of the last things they hit on. Kanebo is now trying to build on this unexpected success



and claims to have succeeded for the first time in culture of tissue from leaves and stems of aromatic geranium. "Bio" perfumes may thus be just around the corner.

The main impetus behind the increased sales of "bio" products — running at three quarters of a million lipsticks a month and outpacing supply — seems to be the allure of "biotechnology" rather than any major improvement in the product. Other Japanese cosmetics manufacturers are now scrambling to join the bandwagon and find their own products. **Alun Anderson**

Nuclear waste

Dump it here, please

THE British nuclear waste disposal agency, NIREX, is facing vocal opposition almost everywhere in Britain to its search for a new low and intermediate level disposal site. But its French equivalent, ANDRA, has had ten enquiries from the mayors of villages in central France inviting it to put its next site in their backyard.

The only problem is that although the French mayors are keen, for reasons of local development and employment, many of their people are not — for fear of the waste. Thus at one site, the village of Neuville-le-roi near Tours on the Loire, the mayor was strongly in favour of ANDRA disposing low and intermediate level waste in the clay subsoil nearby, but his 1,000 villagers mounted a campaign against him. The mayor called a referendum: 63 per cent said no, and ANDRA has now dropped Neuville-le-roi from its list.

However, still on the ANDRA list is Cholet, a town of 60,000 inhabitants south of Nantes and Angers, also near the Loire. Here the mayor is described as "very active" in attracting industry and employment, and the opposition is milder. "The behaviour at Cholet is very different from Neuville-le-roi" said an ANDRA spokesman. The opponents there "are people you can argue with".

One of the arguments is money. The French government, unlike the British, offers local authorities concerned with potential nuclear sites both cash incentives

and cut-price electricity. Thus Cholet stands to gain FF 30 million (£3 million) as a gift for housing and road improvements, and up to FF 1.5 million (£150,000) a year off local electricity bills, if ANDRA chooses it as a disposal site.

In Britain, however, the one named site — at Elstow near Bedford north of London — has met enormous opposition. Bedfordshire County Council has declared the area a "nuclear free zone" and written to the Secretary of State for the Environment, Patrick Jenkin, in strong terms, claiming Elstow "totally unsuitable as a site for a nuclear waste repository". Tempers rose further when the British government decided to allow NIREX to investigate the site by means of a "special development order" — a stratagem which avoids the need for a public inquiry.

The reaction to this British stick was perhaps predictable. The French style, offering a carrot, might have worked better, according to a study made for NIREX by Professor Terrence Lee of the University of Surrey. His survey figures show that 48 per cent of Britons would like monetary compensation (but paid to individuals rather than the council) for the building of a nuclear waste repository nearby, and a further 36 per cent would like compensation paid to the council for community benefit or some similar support — making a total of 84 per cent who say they could be bought off.

Robert Walgate.

Maritime museum plan scuttled

THE *Vityaz*, formerly the pride of the Soviet research fleet, which completed her last voyage in April 1979, is now anchored, a near-derelect with peeling paint and rotting woodwork, off Kaliningrad, *Pravda* reported recently. Plans to turn her into a floating museum have become bogged down in bureaucracy despite backing from ministerial and state bodies.

Vityaz, launched in April 1949, and the third Soviet vessel of her name (meaning "hero" or "champion"), spent most of her working life in the Pacific, carrying geological, ichthyological and meteorological expeditions. Her last, 65th voyage, however, ended with a triumphal tour of the major maritime states, including Italy, France, Spain, Portugal, the United Kingdom and Denmark, before she finally dropped anchor in Kaliningrad. It was originally hoped that the ship could go by canal to Moscow and a future as a museum. In the end, however, it was decided to leave her in Kaliningrad, the major fishing and commercial port of the Soviet Baltic coast. There was talk of a "Museum of the World Ocean" in the centre of the city, on the banks of the canal, with *Vityaz* as the prime exhibit.

But then, in the words of *Pravda*, "it all turned out strangely". *Vityaz*, which had successfully weathered hundreds of thousands of miles of ocean voyaging, somehow became stuck fast when it came to completing the last few metres to her final berth. Now, almost six years after the *Vityaz* dropped anchor in Kaliningrad, only about half the money needed even for the minimum cosmetic refit is available in Kaliningrad. Money has, however, been spent on other things. A special harbour wall for the "memorial ship" has been erected, two smallish pre-war buildings have been renovated for the planned museum, and the surrounding terrain has been artistically laid out. According to the regional Communist Party secretaries, Yu Malakin and P. Kaz'min, public opinion in Kaliningrad still supports the idea of the ship museum, but someone has to make the official decision to build the museum complex. Once it is built, says Mr A. Shkurko, Deputy Minister of Culture of the Russian SFSR, his ministry will be delighted to take it over as a branch of the provincial museum of local history. No one, however, seems willing to assume responsibility for the decision to build.

Vera Rich

Indian science

Gandhi backs research

New Delhi

THE government headed by Rajiv Gandhi, who has pledged to prepare India for the twenty-first century, has allocated the equivalent in rupees of £900 million, or about six per cent of the government's budget for 1985-86, for scientific research and development.

The allocation for science represents only a marginal increase over that for the previous year but it is significant for its emphasis on research and development in non-conventional energy sources, biotechnology, high-power lasers and thermonuclear fusion.

As in earlier years, the lion's share of the budget goes to the Department of Atomic Energy (DAE), which is to set up a new centre for advanced technology at Indore, in Madhya Pradesh. Besides housing a new facility for plasma research, the centre will develop lasers for isotope separation and high power neodymium-glass lasers for laser-induced fusion. DAE also intends to set up a synchrotron radiation source, build superconducting magnets for its heavy-ion

Budget allocations by sector (£ million)

Department of Atomic Energy	£210.0
Space	133.0
Industrial research	114.4
Defence research	104.0
Non-conventional energy	65.0
Agricultural research	55.5
Electronics	78.0
Oceanography	20.7
Meteorology	27.0
Other	93.0
Total	900.6

accelerator and establish a medical cyclotron for producing short-lived positron-emitting isotopes and radioactively labelled pharmaceutical preparations.

The Department of Ocean Development (DOD), which has assessed India's ocean thermal energy conversion (OTEC) potential at 50,000 MW, is planning to begin work this year on a 1-MW OTEC plant on the island of Lakshadweep. DOD will also place an order for an ice-breaker, estimated to cost £30 million, for undertaking expeditions to Antarctica. Previous expeditions were made in vessels chartered from Norway.

Electronics and biotechnology have been singled out as frontier areas. The government has abolished excise duty on computers and drastically reduced customs duty on their import. The Department of Electronics will launch this year a project to design a "fifth generation super-minicomputer incorporating inference and knowledge based management functions". The Ministry of Science and Technology has earmarked a sum of £5 million for a biotechnology centre in New Delhi, its con-

tribution to the UNIDO plan for twin international centres for genetic engineering and biotechnology at Trieste, Italy and in New Delhi.

The Department of Environment has allocated funds for setting up a chain of botanical gardens for building a germ-plasm bank of economic plants, and seed and pollen banks for the conservation of threatened species. The department will also initiate this year a five-year £180-million programme to clean up the polluted river Ganges.

The Department of Space, which gets the second biggest share in the budget, plans to set up five regional remote sensing service centres to facilitate use of aircraft and satellite-sensed data and to provide trained manpower. Images received from Landsat will be augmented with data to be obtained from the French SPOT satellite. The space department will also start work on a new launch-pad for orbiting satellites in polar orbits. The polar launch station is expected to become operational in 1992.

K.S. Jayaraman

UK embryo research

Voluntary authority set up

THE British Medical Research Council (MRC), in association with the Royal College of Obstetricians and Gynaecologists (RCOG), last week replied to the government's inquiry into human fertilization and embryology (the Warnock report). MRC endorses the inquiry's recommendation for a licensing authority for research in these fields, and, recognizing that legislation will take some time, has set up a voluntary licensing authority under the chairmanship of Dame Mary Donaldson and consisting of MRC and RCOG scientists and doctors as well as lay members. The authority would both license experiments and draw up a code of practice. Although agreeing with the Warnock committee's 14-day limit on experimentation on embryos, MRC prefers that the limit be set in terms of stage of embryo development rather than days after fertilization, because of variations in the rates of growth between embryos.

MRC also seeks to address wider issues than those covered by the Warnock report: to define the term "embryo" (a viable conceptus developed from a fertilized egg and not tissue or cell cultures) and that "research" should include "new and untried treatment", a concept excluded in the Warnock report.

Human embryo research was not financially supported at all by MRC before the first British "test-tube" baby was born. Since then, MRC has recognized that research can improve understanding of infertility, may prevent some inherited diseases and congenital abnormalities and may produce safer contraceptive methods. Possible lines of research include:

- Finding the best culture conditions for egg maturation, of help to people suffering recurrent miscarriages after normal conception.
- Tests on defective sperm function, involving egg penetration and subsequent limited development of the fertilized egg. (Up to 50 per cent of human infertility is due partly to male-related disorders.)
- Some 60 per cent of eggs fertilized *in vivo* do not develop beyond implantation because of fetal abnormalities and unknown factors.
- Some congenital abnormalities (for example, Down's syndrome, spina bifida)

probably occur during sperm and egg development.

- Better understanding of the development of chromosomal abnormalities.
- Investigation of infertility caused by production of antibodies to sperm in some women. Such research could lead to a contraceptive vaccine.

The MRC/RCOG report is one of many being submitted to the government. Eventually, legislation will result. But if the Powell bill (see *Nature* 21 February 1985, pp. 612 and 618) becomes law, considered reports such as the MRC statement will be so much wasted paper, for all research and development involving human embryos will be illegal. Techniques such as GIFT (gamete intrafallopian transfer), developed in Texas, where an unfertilized egg is removed from the ovary and immediately transferred to the fallopian tube where it can then be fertilized, would never be developed with such a bill operating. Later this month the first such baby will be born in Britain.

Maxine Clarke

Hazardous chemicals

Congress acts on clean air

Washington

IN the wake of the Bhopal disaster, pressure is mounting in the United States to enforce new limits on emissions of toxic substances from chemical plants.

The effort has been given a recent impetus by a series of revelations from Union Carbide (which operates a methyl isocyanate plant in West Virginia) and other chemical manufacturers concerning accidental and routine venting of hazardous substances. In responding to a survey conducted by the subcommittee on health and the environment under the chairmanship of Representative Henry Waxman (Democrat, California), 67 chemical companies acknowledged venting a total of some 204 chemical substances that the companies themselves identified as "hazardous". Many of the companies had been reluctant in the past to provide this data; Waxman approached the companies directly after

discovering that the Environmental Protection Agency (EPA) had failed to collect this information in connection with its existing programme of regulating hazardous air pollutants.

The subcommittee is still analysing the data and will not release a complete report for several weeks. But a preliminary survey shows that at least a few plants emit surprisingly large quantities of hazardous substances. Borg Warner, for example, reported that one of its plants in West Virginia releases some 8,000 pounds of acrylonitrile, a known human carcinogen, into the air each day.

The subcommittee also found striking variations in emissions between similar plants run by different companies, and even between plants in different states run by the same company.

Under current EPA regulations, only five chemicals are controlled as hazardous air pollutants: vinyl chloride, benzene, asbestos, beryllium and mercury. Congress's environment committees have complained for years about EPA foot-dragging; EPA has listed 27 substances as "suspected hazards" but has not yet decided whether to issue control regulations. Although the legislative history of the 1970 clean air act shows that Congress expected EPA to consider regulating at least 12 specific substances (including chlorine gas, hydrochloric acid, several heavy metals, pesticides and radioactive compounds), the law itself gives the agency broad discretion to decide what constitutes a hazardous air pollutant and what action to take to limit release of pollutants into the atmosphere.

EPA has further provoked Congress by recently proposing a relaxation of controls on emissions of vinyl chloride, a known human carcinogen. Under existing rules, plants that produce vinyl chloride may only vent the chemical through relief valves under emergency conditions. Each time a venting occurs, EPA investigates the incident to determine if it could have been prevented; if it was preventable, the company is subject to civil or criminal penalties. Yet EPA records show that "emergency" venting of vinyl chloride is almost routine; one million pounds was released into the air during the period 1977-80. EPA is now proposing to recognize the fact by allowing each plant four to seven "free" ventings per year; EPA would no longer investigate each incident.

Waxman's subcommittee is working on a bill that would require EPA to produce regulations on a specific list of chemicals and with deadlines incorporated into the legislation. Similar efforts (albeit milder) have been passed by various subsets of Congress as part of proposed revisions of the clean air act, but always to die as the act itself founders on unresolved differences over other issues, notably acid rain controls. Waxman will try to have his bill considered as a separate piece of legislation, not tied to the clean air act.

Stephen Budiansky

Population growth

SIR — The literature, both popular and specialist, often refers to population growth rates as exponential. More often than not this implies merely a rapid rate of increase and not necessarily its proper meaning of a fixed proportional increase in a set timespan. For example, reference to almost any long-term demographic time series shows that the expansion is not truly exponential as it does not result in a straight line on a graph of the logarithm of population against time.

Now let us explore an alternative expansion rate — according to the cube of time since the modern nation was born. Australia is a nation for which the demographic record is known fairly precisely for the non-aboriginal population since its birth in the 1780s, about 200 years ago. There is thus very little flexibility to be tolerated on the birth date of the nation and retrospective analysis

Encouraged by the well-documented Australian example, we might explore others. The figure shows similar relations for data from France², United States³ and Brazil⁴ from which it can be concluded that these nations began in the sense of a modern society in 1233, 1724 and 1783 respectively. Historians may care to comment on events that could justify that conclusion.

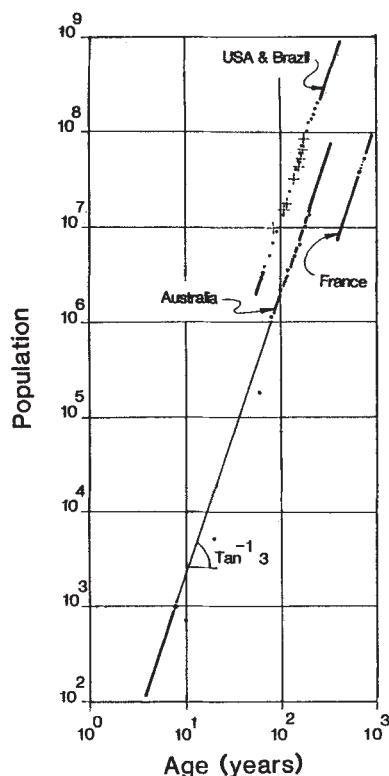
In the meantime, it seems appropriate to draw attention to the far more accurate demographic projections available from this model than from the variably time-dependent exponential expansion models commonly found in the literature. The reader can test this conclusion by

exploring, say, the 50-year forecasts that would have been made at decadal intervals over the past century or so using both this cubic expansion and any exponential model. The results are most encouraging and promise much for demographic planning.

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1. Cameron, R.J. *Official Yearbook of Australia* Vol. 61, 135 (Australian Bureau of Statistics, Canberra, 1976).
2. *Annuaire Statistique de la France* Vol. 88, 25 (Institut National de la Statistique et des Etudes Economiques, Paris, 1983).
3. Teir, J.E. et al. *Statistical Abstracts of the United States* Vol. 100, 6 (US Government Printing Office, Washington DC, 1979).
4. *Anuário Estatístico do Brasil*, 39 (Instituto Brasileiro de Estatística, Rio de Janeiro, 1971).



leads to the initial date of 1780 from the application of this hypothesis to Australian population data¹. In fact, that population generated from the “dumping” there a few years later of several hundred largely undesirable British citizens. The relation shown in the figure for Australia exhibits a general slope of gradient 3, that is, it represents an expansion according to the cube of time on this double logarithmic plot. The only anomalies are in the very early years when the embryonic settlement was subject to reduction by disease in the exotic environment and in the mid-nineteenth century when a gold rush generated an unnatural influx.

Viscosity, noses and IQ

SIR — P.M. Gaylarde’s assertion (*Nature* 313, 425; 1985) that I speculated “that the shape of a person’s nose determines his IQ” is both offensive and inaccurate. In fact, what Gaylarde interpreted as an “attempt to establish spurious grounds for white supremacy” in a manner “as unsavoury as those... used to justify the extermination of Jews and Gypsies in Hitler’s Germany”, was, both in intent and content, exactly the opposite. I urge readers who saw only Gaylarde’s response to read what I wrote before passing judgement (*Nature* 311, 515; 1984).

It is true, as Gaylarde states, that “the viscosity of moist air is less than that of dry air”, but the relevant comparison is between the cool dry air of temperate zones and the hot humid air of the tropics. Unlike liquids, gases gain viscosity with increases in temperature, and that gain more than offsets the reduction from increased humidity. For example, air going from 0 to 40°C and simultaneously from dry to saturated (maximizing the effect of humidity), gains about 20 micropoises of viscosity as a result of increased temperature¹ while it loses about two micropoises of viscosity from increased humidity² — a net gain of 18. Gaylarde is also wrong to think that nose shape, length and hairiness have little effect on airway resistance³. If the length of a tube is doubled, pressure must be increased twofold to maintain constant flow, and if the radius of a tube is halved, pressure must be increased an additional sixteenfold to maintain flow — a 32-fold increase. Add the fact that saturated hot air (say 40°C) contains less oxygen (about 7 per cent less), and one can begin to appreciate the value of a short wide hairless nose in hot humid climates.

But the brunt of my nose argument, the entirety of which was not essential to my point, was that it might be disadvantageous not to have a dust-trapping nose in dusty environments. In support of this speculation I pointed out that “among American racial and ethnic groups, only

blacks and non-Japanese orientals have a conspicuous prevalence of lung disease”. I agree with Gaylarde that lung disease “is related to socioeconomic factors”, but its relative inconspicuousness in hispanics leaves my point intact.

The objective of my contribution was to explain the fact that heritable differences in IQ need not be attributed to differences in “IQ genes”, and I have argued that any heritable difference in IQ between races is especially unlikely to be caused by such genes⁴. My point was that adaptations taken out of their evolutionary context (such as pale thin skin and long, thin, hairy noses in the tropics) are liable to be disadvantageous and could have an incidental effect on IQ. I pleaded that such questions should be investigated because “there are many factors that something could be done about if we knew what they were”, for example, “people with non-dust-trapping noses can have humidifiers in their homes during the winter and take care to wear a dust-mask when doing a dusty job”. And I lamented the fact that “looking for genetic differences that incidentally affect IQ is politically unacceptable”. Gaylarde has proven me right in this last regard.

Misrepresentation of facts, misreading, an astounding failure to comprehend and knee-jerk reply aside, as the son and nephew of men who fought against Hitler’s Germany, and as one who grew up in a home where racists, anti-semites and fascists were clearly “the enemy”, I resent Gaylarde’s ludicrous insinuations.

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1. Weast, R.C. *Handbook of Chemistry and Physics* F-39 (CRC, Cleveland, 1965).
2. Kestin, J. & Whitelaw, J.H. *Humidity and Moisture* Vol. III, 301 (Reinhold, New York, 1965).
3. Negus, Z. *The comparative anatomy and physiology of the nose and perinasal sinuses* (Livingstone, London, 1958).
4. Hartung, J. *Curr. Anthropol.* 21, 131-132 (1980).

The importance of impatience

Unifying gravity with the other three fundamental forces has been brought a large step nearer. But there is a long way still to go.

In a week in which there appears in *Nature* an account of a theoretical framework that may be the best hope yet that theories of particle physics will be united with gravitation, a reminder of how frustrating this problem has become may be in order. The point is nicely illustrated by Dr C. Villi of the University of Padua in the latest issue of *Il Nuovo Cimento* (85A, 175; 1985).

Villi's interest is simple, to explore the question whether the mathematical infinities that have plagued the calculation of particle properties, and which persist, are merely consequences of leaving gravitation out of the calculations. For, as he puts it, "the most significant aspect of the divergences encountered in quantum field theory is that they do not exist in Nature". His method is straightforward: to suppose that true curved space-time is everywhere approximated by flat Minkowski space-time, essentially that of special relativity, from which it follows that the function which defines the approximation at each point of space-time (the form factor) must satisfy Einstein's equation of gravitation.

At this point, another of Villi's discontents comes to the surface. Soon after Einstein first formulated his equations of gravitation (in 1917), he confused the issue by adding to the simplest form admissible on the grounds of geometrical consistency an extra term containing a constant, the gravitational constant. Equations with this extra term are consistent with the physical arguments leading to general relativity, while the presence of the constant made it possible to construct a finite universe containing a uniform distribution of matter whose density was not identically zero.

Villi is scornful of the way the cosmological constant has been dealt with ever since. It is agreed that it must be numerically small, but is it right to follow Pauli (and Einstein's second thoughts) in dropping it altogether? Villi is in no doubt. "No efforts have been made to understand the *raison d'être* [of the cosmological constant]. This is indeed astonishing because its cosmological significance is purely accidental... the parameter would appear in Einstein's equations even if cosmology had never been thought of."

Villi's objective is to show that the cosmological constant can somehow smooth out (with the help of his form factor) the infinities that arise in the calculation of particle properties. Although accepting that the constant is numerically small for large-scale phenomena, he would have it very

large on the scale of particle phenomena.

There are precedents for this line of thinking. One way of making a connection, however tenuous, between gravitational and particle phenomena is to remark that the bending of distant starlight at the limb of the Sun (one of the three accepted tests of general relativity) could otherwise be accounted for if the refractive index of the vacuum were different from unity, which may in turn be explained if the vacuum is polarizable. Villi notes that all attempts to forge such connections have been defeated by objections of doubtful weight.

"In surveying this matter, one is oppressed not only by objective difficulties, but by arguments open to serious doubts and by unnatural assumptions which breed confusion and scepticism. Too many dissertations based on unfruitful and controversial theoretical subtleties have contributed to shattering the problem of the role played by gravity in the subnuclear universe into fragmentary aspects generally contradicting each other... we shall pursue a different line of thought... to overcome the apparent irreconcilability of general relativity with quantum field theory, a dilemma that may provide the seed for a radical rethinking of some physical concepts and of the theoretical schemes... developed."

Fighting talk? Villi confesses that he cannot at this stage provide the general prescription that his programme requires, but goes on to give some telling if particular examples. The form-factor way of regarding space-time as locally flat does get rid of some of the infinities arising in solutions of the Klein-Gordon equation, for practical purposes the generalization of the wave equation for photons to particles with non-zero mass and spin. He thinks it important to tackle the problem in the way in which Feynman first made quantum electrodynamics respectable, by the "path-integral" method, but only after this statement:

"I confess my inability of profiting heuristically from the jungle of theoretical approaches invented in order to transpose to general relativity Feynman's methodology... It is difficult to see a way out of such a puzzle, not only because there are conceptual black corners still unexplored, but also because one does not know how to evaluate the formulae thus obtained, nor how to give them a mathematically precise meaning..." And, having translated Feynman into general relativity which, Villi

warns, "is not very exciting", he has this stirring comment on the way in which field theorists get rid of their infinities:

The "daring manipulation of infinite quantities based on the killing of infinities by counter-infinities raises questions which indicate the need for a thorough revision of the renormalization theory... there remain... inherent mathematical ambiguities in handling divergent expressions... it is a challenging task to find a way towards an alternative formulation of quantum electrodynamics, free from the slaveries of renormalization..."

Where does it all lead? Villi is in no doubt. The vacuum fluctuations beloved of particle physicists must endow empty space with what Einstein called an energy-momentum tensor that differs from zero, and the consequence must be a short-range "cosmological constant" that not merely differs from zero but is very large. And "this has nothing to do" with the large-scale cosmological constant related to the mass-density of the universe at large. Villi's other important general precept is that it may be an illusion on the part of the field theorists to look for a unification on equal terms of the four fundamental forces (strong and weak nuclear interactions, electromagnetism and gravity). Perhaps, he says, gravity always enters on a qualitatively different footing, and should be handled differently from the start.

What M.B. Green (see p.409) has to say notwithstanding, Villi should not be scorned. There is a natural temptation among those who construct theories to account for phenomena not previously well categorized to suppose that only one mathematical framework will accommodate the phenomena, and the trick is to find it. That is how it is at first, but time accretes other ways of solving these problems.

It is too soon for Green to have figured out whether what he has done (and what earlier users of Kaluza-Klein theories, in which redundant "internal" dimensions are collapsed onto ordinary space-time, have attempted) fulfils the objective of Einstein and his later collaborators (Infeld and Bohm, for example) to define the "hidden variables" that would make quantum mechanics determinate. For the time being, it is more important to win some physics from the mathematics. But Villi and his like will continue to be angry if they are forever fobbed off with the answer that their questions cannot be answered in the terms in which they are put. **John Maddox**

Halley's comet

Prospects for Giotto and Halley

from M.K. Wallis

AT the final pre-launch preview*, scientists involved in the Giotto mission to Halley's comet concluded that the risk to the spacecraft from dust particle impacts (see *Nature* 303, 659; 1983 and 305, 756; 1983; *ESA J.* 7, 15; 1983; *Earth, Moon Planets* 30, 31; 1984) is considerably less than had been feared.

Initial pessimism had centred on the likelihood of impacts that would cause a deviation of Giotto's spin axis in excess of 1° , when radio contact would be lost. Although milligramme and smaller-sized grains impacting the protective shield will contribute to the deviation, it is now agreed that the original pessimism was based on an over-weighting of the rather improbable grains exceeding 0.1 g, which might anyway smash through the rear shield and destroy instrumentation.

Predictions of survival are based on predictions of the density and size-distribution of dust particles. The probability of survival against 1° perturbations is now given as around 90 per cent on the nominal dust model for a 500 km flyby distance, although calculations need extending to give a secure guide for future targeting decisions.

Much scientific effort has gone into elaborating models of the comet's dust coma; rather than assuming instantaneous formation, it is now agreed that dust grains can reside in the coma for weeks or even months, forming into anisotropic structures purely because of the comet's motion around the Sun. Model calculations at the NASA-Jet Propulsion Laboratory (JPL) have been extended to encompass long-lived dust grains and are now in agreement with an alternative analysis by ESOC scientists (Fertig, J. & Schwehm, G.H. *ESA Bull.* 38, 36; 1984).

There has been concern about much higher dust densities in jet-like regions. JPL scientists (Sekanina, Z. & Larson, S.M., *Astr. J.* 89, 1408; 1984) have applied digital image-enhancement techniques to photographs of comet Halley's 1910 apparition, and infer enhanced densities, some 10–100 times the mean. It is now accepted, however, that these would be of submicron size (like cigarette smoke) and present a desirable target rather than a threat to the spacecraft's integrity. Study of the jet phenomenon might, alternatively, indicate where to expect the dangerous, slowly-moving, millimetre-sized grains.

Originally, the mission was scheduled to terminate one hour after closest approach, supposing that the spacecraft is unlikely to survive. But there was pressure from experimenters at the meeting to retain trans-

mission options for two days on the outward flight through the extensive gas coma.

The meeting heard that the Pathfinder concept agreed with the Soviet Space Agency is going ahead; data from the Soviet VEGA spacecraft will be used to cut down the error in targeting. If the two VEGAs are tracked by very long baseline radio-interferometry, the error should be under 100 km. Supplementary ground-based astrometry is also to be investigated.

The reserve of one month in the original schedule has now been used up, with final instrument acceptance checks due in late March. No significant problems have arisen on subsystems or experiments during the system test programme, although the

spacecraft as a whole is hotter than predicted, requiring modifications to radiating surfaces. Assembly and final testing of the television camera is running late, so installation and checking procedures have been reorganized around it. One advantageous change to the original schedule gives scientists the option of integrating fully-calibrated versions of their experiments just before delivery of the spacecraft.

The planned launch campaign is due to commence at the beginning of May 1985 with an expected launch date of 2 July 1985 on an Ariane 1 vehicle from the European launch site at Kourou, French Guiana. The optimistic hopes of securing data on the inner coma and pictures of the nucleus from a few hundred kilometres seem likely to be achieved on the basis of this review. □

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Chemical evolution

Origin of biomolecular chirality

from Stephen Mason

THEORIES accounting for the ubiquity of the L-amino acids and the D-sugars in the biochemistry of living organisms centre on mechanisms for the selection of enantiomers of one hand from the heterochiral mixture of a racemic substrate, and for the propagation of optically pure homochiral products. Surprised to learn, in 1953, that the origin of biomolecular handedness still appeared to be problematic, Frank proposed a homogeneous chemical kinetic mechanism for an open flow-reactor system¹. The mechanism contained no internal bias, and the dominance of the L-amino acids and the D-sugars in the natural products, rather than their respective enantiomers, seemed to be wholly a matter of chance. By extending the mechanism and incorporating a recently evaluated internal bias, Kondepudi and Nelson² now define the particular conditions under which a small electroweak advantage factor for the L-series of amino acids and polypeptides³ leads to a determinate choice in the evolution of homochiral biomolecules from an achiral or racemic substrate.

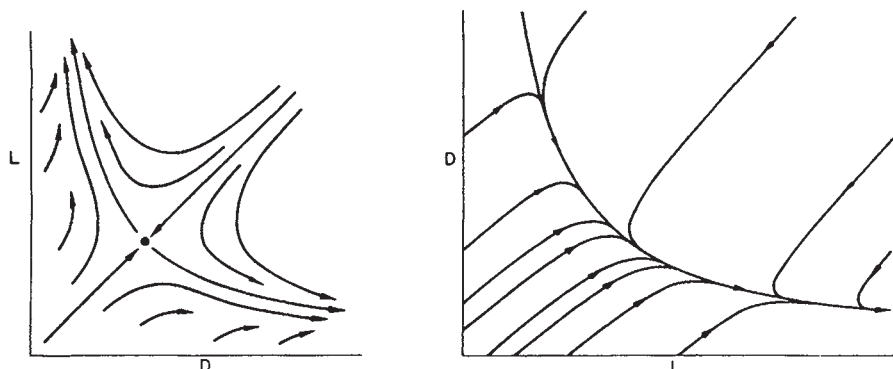
Frank's model avoided the direct intervention of external agencies, such as a chiral field or panspermic celestial seeding, and the various heterogeneous mechanisms advocated in the grand debate on the subject in the correspondence columns of *Nature* during 1898 following F.R. Japp's paper on "Stereochemistry and Vitalism"⁴. Ironically, that correspondence ignored the 1884 conjecture of Pasteur that a dissymmetric force pervades the physical world, extending even to the crystallization dish used for the separation

of enantiomers. The discovery, in 1956, of the non-conservation of mirror-image symmetry in the weak nuclear interaction supported Pasteur's surmise, and the subsequent unification with electromagnetism has allowed the characterization of both the sign and the magnitude of the universal chiral electroweak interaction. The sign is in accord with the observed products of natural selection. The L-amino acids and the L-polypeptides are more stable than their D-enantiomers. But the advantage ratio of the electroweak enantiomeric energy difference (ΔE_{ew}) to the thermal energy (kT) at ambient temperature is only about 10^{-17} (ref.3), too small according to one school of thought⁵ to affect the outcome of biochemical evolution.

Frank envisaged an open reactor system with a continuous input of an achiral substrate from which both enantiomers, L and D, are formed (see figure). Each of the optical isomers autocatalyses its own production and competitively inhibits propagation of its enantiomer. In general, the dynamic generation of a racemic product is metastable and subject to a bifurcation catastrophe. Chance fluctuations sooner or later trigger a kinetic switch to one of the homochiral reaction channels, producing either the L-isomer or the D-enantiomer. Which is produced, in the absence of an internal bias or an external perturbation, depends on the sign of the chance fluctuation¹.

The general solution to the kinetic relationships of the extended mechanism is shown to be a cubic equation in the enantiomeric excess of the handed products, ($\alpha/\beta = ([L] - [D])/([L] + [D])$), where [L]

*European Space Research and Technology Centre, Holland, 19–20 February, 1985.



The time evolution of L and D enantiomers from an achiral or racemic substrate in an open flow-reactor system, with an autocatalytic production of each isomer and an enantiomeric cross-inhibition: left, for identical values of the activation parameters of corresponding enantiomeric intermediates and right, for a small bias favouring the production of the L-isomer. Metastable racemic production is represented by the unique point in the left hand graph, to which either an achiral or a racemic substrate leads, and from which the two homochiral production branches diverge. Modified from reference 1.

and [D] refer to the concentrations of the enantiomers². One root of the cubic equation, correlating with a small input concentration of the substrate into the flow-reactor system, corresponds to a vanishing enantiomeric excess, $(\alpha/\beta) = 0$, giving a racemic product. The other two roots $(\alpha/\beta = +1 \text{ or } -1)$ correlate with high substrate input concentrations, and correspond to the two homochiral reaction branches, giving only the L-isomer or the D-enantiomer. At the transition point, where the racemic production process becomes metastable and bifurcates into the oppositely handed reaction channels, the system develops a magnified hypersensitivity to minor inequalities between the activation parameters of corresponding enantiomeric intermediates in the two homochiral reaction branches. Thermal fluctuations at the temperature, T , of the system randomize and annul any initial trend towards the D or the L production branch unless the difference in activation energy, ΔE , between the enantiomeric intermediates satisfies the relationship $(\Delta E/kT) > 10^{-17}$ (ref. 2).

At ambient temperature, the electroweak advantage ratio for the L-series of amino acids and polypeptides, $(\Delta E_{ew}/kT)$, meets the lower limit of the relationship, so that

selection of the reaction branch for the L-series becomes determinate over a limited range of conditions. With realistic rate constants for the elementary kinetic stages of the model mechanism, the homochiral reaction branch for the L-series is selected with a 98 per cent probability if the passage through the critical hypersensitive transitional bifurcation state is slow. Over 10^4 years this would correspond to an increase in the input concentration of the substrate by 10^{-2} to 10^{-3} moles in a flow reactor with the volume of a small lake, approximately one kilometre in diameter and some four metres deep. This estimate takes into account quantifiable disordering effects, such as the known rates of spontaneous racemization of the amino-acid enantiomers, and the minor enantiomeric photodiscrimination of solar radiation at twilight, when there is a small net circular polarization, oppositely handed at dawn and dusk². □

1. Frank, F.C. *Biochim. biophys. Acta* **11**, 459 (1953).
2. Kondepudi, D.K. & Nelson, G.W. *Nature* **313**, 438 (1985).
3. Mason, S.F. & Tranter, G.E. *Proc. R. Soc. A* **397**, 45 (1985).
4. Japp, F.R. *Nature* **58**, 452 (1898).
5. Morozov, L.L., Kuzmin, V.V. & Gol'danskii, V.I. *Sov. Sci. Rev. D physiochem. Biol.* **5**, 357 (1984).

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Cell biology

Baseless flagellation

from Jeremy S. Hyams

COMPARED with its enigmatic alter ego, the centriole, the basal body is a fairly straightforward organelle. Basal bodies sit, appropriately, on the base of eukaryotic cilia and flagella. Unlike the structures associated with the base of bacterial flagella, they are not actively involved in motility. Rather, they provide the template for the assembly of the 9 + 2 axoneme and, through their association with various rootless structures, anchor the beating cilium or flagellum against the generated torque. This rather comfortable view of

basal body function may, however, have to be re-examined in view of a paper by H.J. Hoops and G.B. Witman on the green alga *Chlorogonium*¹.

In design, the flagellar apparatus of *Chlorogonium* resembles that of its better studied relative *Chlamydomonas*². The two flagella arise from the anterior of the cell in a 'V' configuration. The basal bodies which from the crotch of the V are maintained by a group of striated fibres and serve as the focus for a series of microtubule rootlets that head off towards the

cell's posterior. During forward swimming the two flagella sweep away from each other in a motion which, superficially at least, resembles a human swimmer's breast stroke. Unlike *Chlamydomonas*, where the flagella are resorbed before mitosis, *Chlorogonium* contrives to swim during cell division, the parental flagella remaining attached to the anterior-most daughter cell. Electron microscopy of these dividing cells reveals that the basal-body complex detaches from the flagella to become associated with the poles of the spindle.

Remarkably, not only do the flagella continue to beat in the complete absence of their basal apparatus but the cells still exhibit their normal photophobic and phototactic responses. In *Chlamydomonas*, these responses involve distinct changes in the pattern of flagella beating. During the photophobic response the flagella switch from the asymmetrical ciliary mode used during forward swimming to a symmetrical flagellar waveform that drives the cells backwards³. Phototaxis, on the other hand, involves the inactivation of one flagellum whilst its partner continues to beat, causing the cell to turn⁴.

Experiments on detergent extracts of either isolated flagella or whole cells have shown that both responses are mediated by calcium ions⁴⁻⁶. A rise above 10^{-6} M in the intracellular concentration of calcium triggers the photophobic reaction^{6,7}, whilst a differential response of the two flagella to submicromolar levels of calcium probably underlies phototaxis. The two flagella of *Chlamydomonas* can be distinguished on the basis of their position relative to the asymmetrically positioned eyespot. The flagellum furthest from the eyespot is inactivated below 10^{-8} M calcium whilst the other flagellum continues to beat. Conversely, the flagellum closest to the eyespot is inactivated by 10^{-7} - 10^{-6} M calcium whilst the other flagellum remains active.

By obtaining similar results with dividing *Chlorogonium* cells, Hoops and Witman show that the complex behavioural responses of green algae are intrinsic to the flagella themselves and independent of accessory structures associated with the flagellar base. Taken together with the recent identification of a rhodopsin in *Chlamydomonas*^{8,9}, these new findings suggest that our understanding of tactic responses of eukaryotic microorganisms may soon approach the sophistication of equivalent studies on prokaryotes. □

1. Hoops, H.J. & Witman, G.B. *J. Cell Biol.* **100**, 297 (1985).
2. Ringo, D.L. *J. Cell Biol.* **33**, 543 (1967).
3. Boscor, J.S. & Feinleiv, M.E. *Photochem. Photobiol.* **30**, 499 (1979).
4. Kamiya, R. & Witman, G.B. *J. Cell Biol.* **98**, 97 (1984).
5. Bessen, M., Fay, R.B. & Witman, G.B. *J. Cell Biol.* **86**, 446 (1980).
6. Hyams, J.S. & Borisy, G.G. *J. Cell Sci.* **33**, 235 (1978).
7. Schmidt, J.A. & Eckert, R. *Nature* **262**, 713 (1976).
8. Foster, K.W. et al. *Nature* **311**, 756 (1984).
9. Berg, H.C. *Nature News and Views* **311**, 702 (1984).

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Astronomy

Direct estimate of Hubble's constant from the double quasar

from C. Martin Gaskell

DETERMINATION of the time delay between observed variations in the two images of the first gravitationally lensed quasar to be discovered (0957+561 or 'the double quasar') has enabled Hubble's constant (H_0) to be estimated directly¹. This way of measuring H_0 short-circuits the many intermediate steps in 'classical' estimations but, unfortunately, is very model dependent.

The double quasar was discovered by D. Walsh, R.F. Carswell and R.J. Weymann as a pair of quasars, 6 arcseconds apart, with virtually identical spectra². Subsequent research verified their initial guess that these two images come from one and the same quasar: the gravity of a galaxy in the line of observation is bending the light and producing two images of the more distant quasar³. Long before this system was discovered it had been pointed out that variability of a gravitationally lensed object would allow an estimate of the distances involved and hence of H_0 , the constant that relates the velocity of recession of the galaxy or quasar to its distance ($1/H_0$ is the approximate age of the Universe)⁴. In essence, the method is simple: if the shape of the various light paths is known, then the time delay due to the difference in light travel time) between seeing a given variation in one image and the other, gives the scale size. (For the double quasar there are actually three images, but the third is very faint.)

Like virtually all quasars⁵, 0957+561 was quickly shown to exhibit 'flickerings' in brightness (of 30 per cent or so) that last weeks or months⁶. Once that was known, a number of observatories mounted photometric monitoring programmes, and, while waiting for the results, theoreticians refined the model of the mass distribution in the 'lensing' galaxy and the cluster in which it is located^{7,8}. The expectation was that variability of the northern image would precede variability of the southern image by a couple of years.

R. Florentin-Nielsen and K. Augustesen of Copenhagen University Observatory were among the observers following 0957+561. They took repeated photographs with a 20-inch Schmidt camera, a small telescope by modern standards, and in much poorer sky conditions than United States' observers, but with the advantage of being far enough north to follow the double quasar all year round. In early 1980, the southern image brightened while the northern one stayed constant; the southern image was presumably showing some event that could have been seen a year or two earlier in the northern image. Both images then stayed constant in brightness until

1982 when the northern image started to brighten by 30 per cent while the southern image stayed constant. In 1983, the northern image started to fade. There had been a clear 'event'. It was then just a matter of waiting until the southern image followed suit for an estimate of H_0 to be made. By July 1984 it was clear to Florentin-Nielsen that the long-awaited rise in the southern image had begun. In his recent paper¹, he estimates the time delay to be 1.55 ± 0.1 years and derives H_0 as $77 \text{ km s}^{-1} \text{ Mpc}^{-1}$ corresponding to a Hubble age ($1/H_0$) of 13×10^9 years.

How reliable is this estimate of H_0 ? The formal observational error is only $\pm 5 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Classical astronomical methods (for example, using Cepheid variable stars) currently yield H_0 values of 43–95 $\text{km s}^{-1} \text{ Mpc}^{-1}$, and a recent review favours 68 or 82 $\text{km s}^{-1} \text{ Mpc}^{-1}$, depending on the methods used. The latest work on type I supernovae¹⁰ favours a value of 58 $\text{km s}^{-1} \text{ Mpc}^{-1}$. The Florentin-Nielsen value is clearly well within the range under debate.

Within a year or two the observational uncertainty in the time lag should have been narrowed down by a factor of two or more. But the real uncertainty is greater. To calculate H_0 , Florentin-Nielsen relies on detailed modelling by Borgeest and Refsdal⁸ of the mass distribution in the

lensing galaxy and the cluster that contains it. The question is whether Borgeest and Refsdal have correctly modelled the influence of the cluster on the estimate of H_0 . They believe it is less influenced by the cluster parameters than had been feared by, for example, Dyer and Roeder⁷, and that the effect of the cluster galaxies might be leading to a slight overestimate of H_0 from the double quasar. Nevertheless, it remains the largest source of error.

Fortunately, the free parameters in the mass distributions may soon be narrowed down by radio observations of very high spatial resolution that are underway. The models of the mass distributions will have to match the twisting and distortion seen in the radio structure. If they do, then there is some confidence that the time delays, and hence H_0 , have been calculated correctly. Meanwhile, optical observers will be calmly continuing to follow the two images to ensure that the current outburst in the southern image really matches the 1982–3 record for the northern image. If not, some major rethinking will be necessary. □

1. Florentin-Nielsen, R. *Astr. Astrophys.* **138**, L19 (1984).
2. Walsh, D., Carswell, R.F. & Weymann, R.J. *Nature* **279**, 381 (1979).
3. Young, P.J., Gunn, J.E., Kristian, J., Oke, J.B. & Westphal, J.A. *Astrophys. J.* **241**, 507 (1980).
4. Refsdal, S. *Mon. Not. R. astr. Soc.* **128**, 307 (1964).
5. Angione, R.J. & Smith, H.J. in *IAU Symposium No. 44 External Galaxies and Quasi-Stellar Objects* 171 (Dordrecht Reidel 1972).
6. Keel, W.C. *Astrophys. J.* **255**, 20 (1982).
7. Dyer, C.C. & Roeder, R.C. *Astrophys. J.* **241**, L133 (1980).
8. Borgeest, U. & Refsdal, S. *Astr. Astrophys.* **141**, 318 (1984).
9. van den Bergh, S. *Q. J. R. astr. Soc.* **25**, 137 (1984).
10. Arnett, W.D., Branch, D. & Wheeler, J.C. *Nature*, **314**, 337 (1985).

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Parasitology

Towards schistosomiasis vaccines

from F.E.G. Cox

SCHISTOSOMIASIS, one of the world's major diseases, is caused by adult worms that inhabit blood vessels associated with the lower intestine or bladder. The worms survive in these immunologically exposed sites largely because while migrating there as juveniles they acquire host-like surface antigens from the skin, where they enter the body. So effective is this disguise that it is not at all clear if there is any effective immunity in man¹. Vaccines consisting of irradiated juvenile worms² have been the basis of successful field trials in cattle in the Sudan³ and China⁴ but are unlikely to be acceptable for human use. Therefore, several groups have investigated the potential of extracts of juvenile and adult worms. The results are disappointing, although some purified surface antigens have been used with limited success in mice and rats. The most recent study indicates that some protection can be induced in monkeys⁵. This is a major step forward.

Maire Smith and John Clegg, of the Wellcome Research Laboratories, have identified and purified two surface antigens associated with both juvenile and adult worms⁵. The larger molecule, given twice with alhydrogel adjuvant, induces partial protection both in mice and cynomolgus monkeys. The smaller antigen, which could be extracted from the adult worms only with difficulty, also protects mice. These results are promising in that antigens common to the surfaces of both juvenile and adult worms have some protective capacity, but disappointing in that the levels of protection are low and inconsistent. Nevertheless, they indicate that the approach of identifying and purifying surface antigens is worth pursuing. The next step is to clone the genes encoding these molecules, to express them and to test the products.

Although it is now possible to predict future directions of research, it would be

foolish to assume that all is now plain sailing. André Capron and his colleagues in Lille have shown that an IgG2a antibody is involved as a bridge between a schistosome surface antigen and the eosinophils that act as killer cells in the protection of rats against schistosomiasis⁶. More recently, however, they have shown that the same antigen can also elicit the production of an IgG2c antibody, which actually blocks the protective activity of the IgG2a⁷. In developing a vaccine based on purified antigens, therefore, it will be essential to ensure that the antibodies produced do not actually block some unrecognized protective mechanism. Similarly, care will have to be taken to ensure that a vaccine does not result in the death of the migrating juveniles in an unsuitable part of the body. In rats immunized with irradiated juveniles, the worms in the vaccine die in the skin or lungs, whereas those of a challenge infection die during migration from the lungs to the liver⁸. The dangers of the release of antigen in the lungs or liver are obvious, placing yet another constraint on the development of a vaccine.

The relative lack of success in vaccinating animals, especially primates, against schistosomiasis has led some workers to investigate less conventional methods, including the induction of non-specific immunity by the use of immunostimulants. Results obtained in baboons have been equivocal but do suggest that non-specific activators of macrophages and monocytes can provide some protection against schistosomiasis⁹. Overall, specific and non-specific immunization can at best only reduce the worm burden in the host to about half, which may be of advantage to the individual but not to the community, as it would have a negligible effect on transmission.

Some may argue that a vaccine against schistosomiasis is not really necessary because excellent drugs are available. But the first warnings have already been sounded that this situation may not last for long. Praziquantel is an extremely effective and safe drug, albeit somewhat expensive, but several serious side effects have been reported recently from a small community in Zaire¹⁰ and it must be assumed that similar reports will be forthcoming from elsewhere. Both chemotherapy and vaccination have advantages and disadvantages. Ideally, a programme of schistosomiasis control would incorporate the best of both approaches, with health education and the use of molluscicides. □

1. Butterworth, A.E. *et al. Immun. Rev.* **61**, 5 (1982).
2. Bickle, Q.D. *et al. Parasite Immun.* **5**, 499 (1983).
3. Majid, A.A. *et al. Am. J. trop. Med. Hyg.* **29**, 452 (1980).
4. Hsu, S.Y. *et al. Am. J. trop. Med. Hyg.* **32**, 367 (1983).
5. Smith, M.A. & Clegg, J.A. *Science* **227**, 535 (1985).
6. Grzych, J.M. *et al. J. Immun.* **129**, 2739 (1982).
7. Grzych, J.M. *et al. J. Immun.* **133**, 998 (1984).
8. Ford, M.J. *et al. Parasitology* **89**, 461 (1984).
9. Sturrock, R.F. *et al. Parasitology* **90**, 101 (1985).
10. Polderman, A.M. *Trans. R. Soc. trop. Med. Hyg.* **78**, 752 (1984).

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Palaeontology

Outlines of the distant past

from Peter Gambles

THE dawn of life was also the dawn of death. Since the remote Precambrian, more than 3,500 million years ago, countless plants and animals have died, but only an incredibly small fraction have been preserved in the rocks as fossils. Yet it is this meagre record that charts the path of evolution.

Most people will be familiar with the more common forms of preservation, such as sea-shells in limestones or the great mineralized skeletons of dinosaurs in museums. Although palaeontologists can glean a lot of information from the morphology of these 'hard parts' of animals and from their tracks and marks (known as trace fossils), ideas concerning their appearance and behaviour when alive are difficult to substantiate. But every once in a geological while, circumstances combine to yield fossils with exquisitely preserved outlines of 'soft parts' — limbs and internal organs. Extraordinary fossil biotas of that type were the subject of a recent meeting at the Royal Society*.

Many of the specimens recovered are objects of (sometimes morbid) interest in their own right. The structure of the leg of a trilobite from the Burgess Shale (Fig. 1), which died 530 Myr ago, or the outlines of the feathers of *Archaeopteryx*, help relate these creatures to their modern counterparts. Other finds are so outlandish they can barely be classified (indeed, one has been called *Hallucigenia*).

Trace fossils of crawling animals, such as horse-shoe crabs, are not uncommon. But in the Solenhofen limestone (150 Myr) a dead animal is found at the end of the tracks. Elsewhere there are insects trapped in amber, mammoths in ice, even a mother ichthyosaur dead in childbirth. It seems that an essential requirement for this exceptional preservation is that the environment suddenly becomes inimical to life. This can happen when there is rapid deposition of a smothering blanket of mud

seafloors, or if creatures fall or are swept into an anoxic trap. The Burgess shale formed in such a trap at the base of a Cambrian reef.

Perhaps of even greater significance is the preservation of wholly soft-bodied organisms. About 70 per cent of modern biota is soft-bodied and S. Conway Morris (Cambridge University) asked whether this proportion has varied with time. Effectively no shells or exoskeletons have been preserved from before the so-called Cambrian evolutionary explosion. But detailed studies of the Cambrian Burgess Shale fauna suggest that there has been little change in the proportion of soft-bodied biota over the past 600 Myr. Put another way, more than two thirds of the original biota are not represented in most 'good' fossil localities, so any fossils of soft-bodied organisms may help fill large gaps in our understanding of the ecology of ancient environments. As an example, there has seemed to be a lack of predators in 'typical' shelly Cambrian faunas, which has led some workers to believe that the world was then a tamer place. But, according to Conway Morris, several soft-bodied organisms from the Shale look decidedly ferocious; their preserved gut contents identify them as predators.

*'Extraordinary fossil biotas: their ecological and evolutionary significance', Royal Society, London, 20-21 February 1985.

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Fig. 1 The trilobite *Olenoides serratus* from the Burgess Shale, showing details of the legs and gills (H.B. Whittington, Cambridge University).

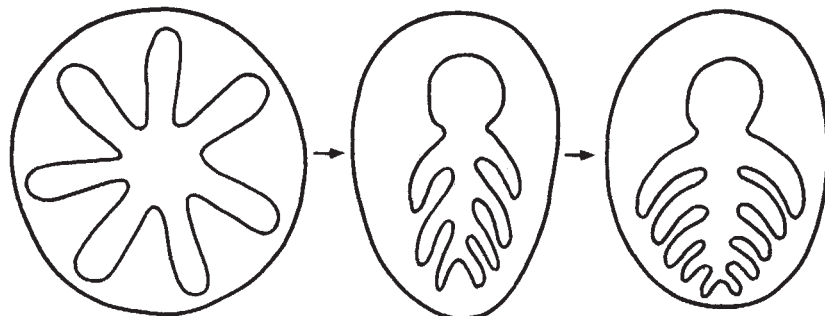


Fig. 2 Evolutionary progression of the metazoan bodyplan from odd-number radial symmetry through "glide reflection" to true bilateral symmetry (M. Fedonkin). See text for details.

A question that arises out of this reappraisal concerns the role of soft-bodied organisms in the evolutionary radiation of the early Cambrian. Many of the more enigmatic fossils seem to represent body plans that are not exhibited by any living creature. The present-day Metazoa (multicellular animals) are dominated by bilaterally symmetrical body plans, often with a degree of segmentation. The coelenterates, with concentric or radial symmetry, today constitute only a small part of the animal kingdom. But M. Fedonkin (USSR Academy of Sciences, Moscow), studying the soft-bodied biota from the Vendian (about 650 Myr) of the White Sea Russian Platform and polar Siberia, concludes that ≥ 70 per cent of animals were then radially or concentrically symmetrical; those with bilateral symmetry are often totally absent.

Some of the more bizarre forms exhibit radial symmetries that today are very rare or non-existent. Thus, an homogeneous group with primary 3-fold symmetry is recognized, and forms with 4- and 7-fold ordering are also common. A particularly important group, according to Fedonkin, is that in which the symmetry remains

radial, but the order (number of arms, gastral cavities, and so forth) actually increases during growth. A possible insight into metazoan evolution comes from fossils such as the late Precambrian *Dickinsonia* which at first sight are bilaterally symmetrical but on closer examination have different numbers of segments on the left- and right-hand sides. Fedonkin terms this phenomenon "glide reflection" and believes that such forms would not be dynamically stable and so evolved to become properly bilateral. He envisages a major evolutionary pattern, recorded in the Vendian rocks, of a gradual change from the dominance of concentric and radial symmetry and glide reflection, to bilateral segmentation (Fig. 2). A. Seilacher (Universität Tübingen) and others dispute this view, finding no evidence that *Dickinsonia* had a mouth or a gut or any semblance of a metazoan digestive system. Consequently they believe such extraordinary fossils represent not the dawn of metazoans but perhaps a completely different kingdom. □

Peter Gambles is an assistant editor of *Nature*.

Nitrogen fixation

Differentiating cyanobacteria rearrange their *nif* genes

from William D.P. Stewart

CYANOBACTERIA (blue-green algae) are oxygenic photosynthetic prokaryotes, many strains of which fix N_2 and which probably dominated the Earth's biota during the middle Precambrian, 2,500–570 million years ago. On page 419 of this issue, J.W. Golden, S.J. Robinson and R. Haselkorn report that, despite their ancient origin, the N_2 -fixing photosynthetic cyanobacteria possess a molecular complexity that belies their morphological simplicity¹.

Although long regarded as algae, mainly because of their pigment composition and mode of oxygenic photosynthesis, cyanobacteria are probably more akin to prokaryotic bacteria than to eukaryotic chlorophyllous plants, and are seldom still classified as blue-green algae. They are most noticeable in extreme environments,

for example in parts of Antarctica where they may form 'algal peat', in hot desert regions including parts of the Sahel, on bare rock surfaces, in hot-spring regions and in living stromatolites. Cyanobacteria are also important components of the marine phytoplankton and they, or more probably their colourless analogues, are components of the microflora of the hydrothermal vent regions of the Galapagos Rift. In many parts of the world, but particularly in south-east Asia where fertilizer nitrogen is not readily available, cyanobacteria are important providers of biologically fixed nitrogen for the growth of the rice plant^{2,3}.

It is partly because of their unique ability to fix N_2 while photosynthesizing in the manner of higher plants that the cyanobacteria have recently aroused the

attention of molecular biologists. Their study may, in the long term, have relevance to the possibility of introducing the genes responsible for N_2 -fixation (the *nif* genes) into the plastids, whether chlorophyllous or not, of higher plants. They have also gained attention as possibly useful models for developmental molecular biologists because many forms occur as simple unbranched filaments with a maximum of three cell types: heterocysts, the sites of N_2 fixation; akinetes, which are perennial; and vegetative cells, from which akinetes and heterocysts develop⁴. Haselkorn and co-workers now add to the scientific attractions of cyanobacteria by showing that they possess a so-far unique capacity to rearrange some of their N_2 -fixing genes, notably two that encode the major components of nitrogenase.

The mechanism that allows the O_2 -sensitive nitrogenase to function in oxygenic cyanobacteria such as *Anabaena* was for long unknown. It was then discovered^{5,6} that the peculiar, empty-looking heterocysts⁷, which occur in most N_2 -fixing cyanobacteria, are the loci of nitrogenase activity in air and in the light, and that by various biochemical modifications, they provide an anaerobic micro-environment in which nitrogenase is synthesized and is functional.

A drawback, until recently⁸, to the detailed genetic analysis of N_2 -fixation and heterocyst production in cyanobacteria has been the fact that although mutants of cyanobacteria are readily obtainable, there has existed no good system for the transfer, in the laboratory, of genes into heterocystous cyanobacteria. Thus, genetic analysis by complementation of cyanobacterial mutants has not been possible. An alternative approach, used by Haselkorn and colleagues, is to use the *nif* genes of the enteric bacterium *Klebsiella* as probes for those of *Anabaena*. In *Klebsiella* there are 17 *nif* genes, organized into seven or eight transcriptional units and arranged in a cluster occupying about 23 kilobases of DNA and located near the genes for histidine biosynthesis (see ref. 9). The genes that encode the major nitrogenase components — the iron protein and the iron-molybdenum protein — are *nif K*, *nif D* and *nif H*. The iron protein is composed of two identical subunits, both encoded by *nif H*. The iron-molybdenum protein contains one pair of identical subunits encoded by *nif K* and another by *nif D*. Although the two proteins do not fix N_2 alone, in combination they may do so in the presence of a source of reductant, Mg^{2+} and ATP, and in the absence of O_2 (see ref. 10).

Haselkorn and his colleagues have already shown that, while the *nif K*, *D* and *H* genes are clustered in *Klebsiella* and some other N_2 -fixing organisms, in DNA extracted from filaments of *Anabaena* 7120 the *nif D* and *nif H* genes are contiguous but separated from *nif K* by about 11 kilobases. The Chicago group has

now compared the arrangement of the *nif K*, *D* and *H* genes in whole filaments of *Anabaena* with that in heterocysts during their differentiation from vegetative cells. While confirming that *nif K* is separated from *nif D/H* in vegetative cells, they show that a gene rearrangement occurs within the heterocyst at a late stage in differentiation. What happens is that the 11-kilobase intervening DNA sequence between *nif D/H* and *nif K* is excised, so that the three genes become clustered and function as a single transcriptional unit, with nitrogenase structural proteins being synthesized, and nitrogenase activity commencing, within the heterocysts. The excised portion remains in the heterocyst as a circular molecule of unknown function.

These findings are of interest not only because they are unique but because they emphasize the usefulness of cyanobacteria for the study of differentiation. The heterocysts of cyanobacteria such as *Anabaena*, show a distinct spatial pattern; between 8 and 10 per cent of the total cells along a filament are heterocysts and new heterocysts develop equidistantly from two existing heterocysts. That is, in some as yet unknown way, existing heterocysts may regulate the spacing pattern of new heterocysts (there is no evidence that nitrogenase is involved in the process). Furthermore, in organisms such as *Nostoc* 7524 there is evidence that with certain external (and no doubt internal) conditions, a cell that normally develops

into a heterocyst develops instead into an akinete¹². The molecular events leading to the cessation of cell division and determining whether a differentiating cell regresses, or further differentiates, and if so into which cell type, is an intriguing area of molecular biology that has relevance not only to nitrogen fixation but to our basic understanding of cellular developmental biology. At a time when the UK Agricultural and Food Research Council is examining N_2 -fixation research, the new results with cyanobacteria will, perhaps, emphasize the merits of scientific diversity in this field. □

1. Golden, J.W., Robinson, S.J. & Haselkorn, R. *Nature* **314**, 419 (1985).
2. Fogg, G.E. *et al. The Blue-green Algae* (Academic, London, 1973).
3. Stewart, W.D.P. *A. Rev. Microbiol.* **34**, 497 (1980).
4. Carr, N.G. & Whitton, B.A. (eds) *The Biology of Cyanobacteria* 2nd edn (Blackwells, Oxford, 1982).
5. Stewart, W.D.P., Haystead, A. & Pearson, H.W. *Nature* **224**, 226 (1969).
6. Bothe, H. *et al. in Advances in Nitrogen Fixation Research* (eds Veeger, C. & Newton, W.E.) 199 (Nijhoff/Junk, The Hague, 1984).
7. Fogg, G.E. *Ann. Bot.* **13**, 241 (1949).
8. Volk, C.P. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1561 (1984).
9. Dixon, R.A. *J. gen. Microbiol.* **130**, 2745 (1984).
10. Ludden, P.W. & Burris, J.E. (eds) *Nitrogen Fixation and CO_2 Metabolism* (Elsevier, New York, 1985).
11. Haselkorn, R. *et al. Ann. Microbiol. (Inst. Pasteur)* **134B**, 181 (1983).
12. Sutherland, J.M., Herdman, M. & Stewart, W.D.P. *J. gen. Microbiol.* **115**, 273 (1979).

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Scale-invariant phenomena

Viscous fingers and fractal growth

from L.M. Sander

A RECENT article in *Nature* by Nittmann, Daccord, and Stanley¹ is a particularly instructive example of the power of basic science to find connections between apparently unrelated natural phenomena. It is an experimental account of the shape of the interface between two fluids with relevance to oil-field technology. The shape is shown to belong to a class that includes the shapes of electrolyte deposits^{2,3}, metals and dielectric breakdown structures such as lightning⁴. The common feature of the objects is that they are all scale-invariant fractals of the same fractal dimension⁵.

The experiment of Nittmann *et al.* deals with the long-known fact that when a relatively non-viscous fluid is forced against a viscous fluid an instability results: 'fingers' of the less viscous fluid intrude into the other. For example, when carbon dioxide is pumped into an oil field, the efficiency of oil recovery is not very large because instead of the oil being pushed uniformly across the field, it is broken up by the branching pattern of the fingers. The usual laboratory realization of the system is a Hele-Shaw cell: water is pumped into a long channel in which oil is trapped be-

tween closely-spaced parallel plates. In the essentially two-dimensional region between the plates, fingering is easily seen.

In past experiments, the shape of the fingers has been dominated by the interfacial tension between the two fluids, which tends to favour coarse fingers. Typically, one finger is formed with a width near to that of the cell. But, in an oil field, the region of interest is much bigger than a single finger. To simulate the oil field in their experiments, the essential step of Nittmann *et al.* was to use two fluids whose interfacial tension is essentially zero — water and an aqueous polymer solution that has a slow rate of mixing with water. In addition, the polymer solution has very high effective viscosity. Both these factors favour the formation of small fingers.

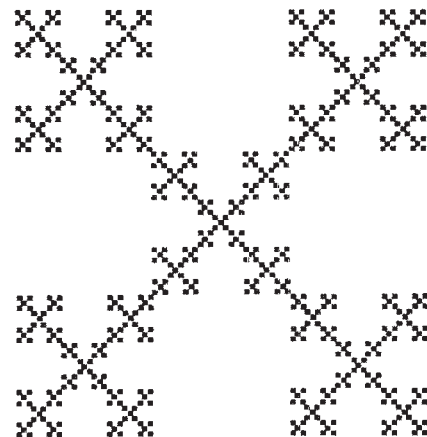
It is with respect to the sprawling, many-branched structures formed as the small fingers of water advance into the polymer solution that connections can be made to disparate phenomena. For, as Paterson has pointed out⁷, the fundamental instability in this situation is the same as in other systems where diffusion limits growth. Here, fingers grow because it is easier for viscous

fluid to flow away from a sharp tip than a flat interface. In dielectric breakdown, the electric field is largest at the end of the breakdown channel, leading to further extension at the point. In electrolyte deposition, new ions more easily find sharp tips than flat areas; the tips thus extend.

For instability dominated growth, the unifying framework is the diffusion-limited aggregation (DLA) model⁸. In this idealization of growth by diffusion, which is used in computer simulations, a particle walks randomly until it finds a nucleation centre, where it sticks. Then another particle is released and walks until it finds either the centre or the other particle and sticks. And so on. The remarkable result of the simulations is that the structures they form are scale-invariant and fractal.

Scale-invariant objects look the 'same' at any magnification. The disorderly structures already mentioned share this property with other natural objects, such as coastlines. The figure gives a simplified example, generated by an obvious rule, which looks the same at a discrete set of magnifications. In order to see what is meant by a fractal, we define D , the fractal dimensionality⁵. Cover the object in the figure with a set of disks of radius r . This takes a certain number of disks, $N(r)$. Now change r and see how N changes. Almost always we find $N(r) = Cr^{-D}$ where C is a constant. For a straight line, $D=1$ and for a square, $D=2$; in these cases D corresponds to our usual ideas of dimensionality. But for the illustrated figure, dividing the radius by 3 multiplies N by 5. Thus $D = \log(5)/\log(3)$. An object whose D is not given by its topology is a fractal.

Electrolyte deposits, dielectric breakdown structures and DLA clusters all have a measured D of about 1.7 in two dimensions of space. Their fractal properties can be studied using the simple DLA model. The fingers of Nittmann *et al.* have a smaller D , but the authors show that this is probably the effect of the finite width of their channel. All of these structures belong to the same universality class which seems to be that associated with growth far from equilibrium. Another class, corresponding to DLA in three dimensions (with $D=2.4$), has been seen in electrolyte deposition². Still another class appears in



the description of flow through porous materials — 'the percolation problem'.

The experiment of Nittmann *et al.* is also significant in that it gives insight into the minimum requirements of a DLA-like structure. Although DLA seems quite simple, it has defied complete analysis. A major point of contention is whether noise due to the discrete nature of the particles making up the cluster dominates the dynamics. Dielectric breakdown structures have links of more-or-less fixed length and electrolytic deposits are polycrystalline. Experiments on these systems shed no light on the question. But the fluid system of Nittman *et al.* is continuous. Apparently, discreteness is not essential to make a DLA-like fractal. There has been speculation that this would indicate that DLA is an example of chaos. In practical terms it may mean that solidification far from equilibrium, which can also be mapped onto DLA, could give rise to fractals — for example, if a snowflake were allowed to grow very large, it would probably be fractal, too. □

1. Nittmann, J., Daccord, G & Stanley, H. E. *Nature* **314**, 141 (1985).
2. Brady, R.M. & Ball, R.C. *Nature* **309**, 225 (1984).
3. Matsushita, M., Sano, M., Hayakawa, Y., Honjo, H. & Sawada, Y. *Phys. Rev. Lett.* **53**, 286 (1984).
4. Niemeyer, L., Pietronero, L. & Wiesmann, H. J. *Phys. Rev. Lett.* **52**, 1033 (1984).
5. Mandelbrot, B. *The Fractal Geometry of Nature* (Freeman, San Francisco, 1982).
6. Saffman, P.G. & Taylor, G.I. *Proc. R. Soc. A* **245**, 312 (1958).
7. Paterson, L. *Phys. Rev. Lett.* **52**, 1621 (1984).
8. Witten, T.A. & Sander, L.M. *Phys. Rev. Lett.* **47**, 1499 (1981); *Phys. Rev. B* **27**, 5685 (1983).

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100 Years Ago

THE following account, we learn from *Science*, of unusual phenomena was received, March 10, at the Hydrographic Office, Washington, from the branch office in San Francisco. The barque *Innerwich*, Capt. Waters, has just arrived at Victoria from Yokohama. At midnight of February 24, in latitude 37° north, longitude 170° 15' east, the captain was aroused by the mate, and went on deck to find the sky changing to a fiery red. All at once a large mass of fire appeared over the vessel, completely blinding the spectators; and, as it fell into the sea some fifty yards to leeward, it caused a hissing sound, which was heard above the blast, and made the vessel quiver from stem to stern. Hardly had this disappeared when a lowering mass of white foam was seen rapidly approaching the vessel. The barque was struck flat aback; but, before there was time to touch a brace, the sails had filled again, and the roaring white sea had passed ahead. The master declares that the awfulness of the sight was beyond description and considers that the ship had a narrow escape from destruction.

From *Nature* **31** 514, 2 April 1885.

Oceanography

Sonar classification of sea beds

from M.L. Somers

ALTHOUGH side-scan solar is used widely in both scientific and engineering investigations of the sea bed, the task of extracting a numerical classification of sea beds from the backscattered sound has proved intractable. But an experienced human observer who has had access to the results of comparisons between sonographs and physical samples can rapidly make accurate qualitative classifications, so there must be some quality of the backscattering that gives the necessary clues. On page 426 of this issue, Z. Reut, N.G. Pace and M.J.P. Heaton offer a solution to the problem and a method of classification into six sea-bed types — mud, sand, clay, gravel, stones and boulders.

The backscattering of sound from the sea bed is a complicated function of the grazing angle of the sonar signal and the roughness of the sea bed, with further complications thrown in by the acoustic impedance of the sea-bed material: if the ratio of sea bed to water impedance is close to unity (as in the case of mud), then there is appreciable penetration of sound into the sea bed and the possible production of a boundary wave, which can interact with the incident wave, profoundly modifying the backscattering process (see Somers, M.L. and Stubbs, A.R. *IEE Proc.* **131**, Pt F; 1984). To circumvent these problems, Reut *et al.* have concentrated on the rate of fluctuation of the backscattered sound over short periods of time. Their method is based on the observation that the fluctuations generally become more rapid as the ground gets rougher. There are, of course, large differences in total energy, and the peak-to-valley ratios can actually be greater over a soft or smooth sea bed than over a rough one, but the rate of fluctuation is still generally less than if the sea bed is rough.

The method requires fairly careful normalization, for which Reut *et al.* have chosen to use cepstrum analysis, an outline of which is given in their paper. By taking the mean of the power spectrum, all considerations of phase can be ignored, so ensuring that when the inverse transform is taken the result peaks at, or near to, zero time delay. The effect of taking logs before doing the inverse transform is to de-emphasize any d.c. level in the signal, which shows up as a large peak at zero frequency in the power spectrum. The two-power cepstrum integral parameters give a measure of how closely the cepstrum is confined to low time-lag values.

Justification of the method comes both from the foregoing qualitative arguments that can be mustered in its favour and from the fact that it seems to work — 120 sea-bed areas are correctly classified into the six types with the only overlap being bet-

ween gravel and stone types. A similar result could probably be obtained by applying automatic gain control, removing the mean and taking the auto-correlation function, though this would possibly be computationally less attractive.

It would be interesting to see the method applied to a wider selection of sea-bed echoes, using a number of frequencies instead of the single 48-kHz frequency used by the authors. An application which comes to mind concerns the problem of mapping and assessing fields of manganese nodules in oceans that are several thousand metres deep. This will require low-frequency sound, such as used in the long-range side-scan sonar, GLORIA II. Manganese nodule fields occur very widely in the Abyssal Plain regions of the deep ocean and should be visible on a GLORIA record. If so, it might at least be possible to delineate the boundaries of the fields, and perhaps even to give a measure of nodule abundance.

Side-scan sonar requires oblique incidence for its best visual effect, so that depth/range ratios need to be less than about 0.2. But most systems working from the surface in deep water, or at high frequencies in shallow water, operate at near-vertical incidence and the backscattering process is somewhat different. It will be interesting to see how the classification system of Reut *et al.* performs in such circumstances.

Turning from their concern with problems of propagation and surface reverberation, the underwater-acoustics community is devoting increasing attention to sonar scattering. Many theoretical papers and some reporting measurements or equipment will be given at the International Conference on Scattering Phenomena on 2-3 April at the Admiralty Research Establishment, Portland, UK. Various applications of the analysis of sea-bed backscattering, both civil and military, are being explored. Of the civil applications, many involve the estimation of resources. Manganese nodules have already been mentioned. Gravel provides a second example. Over ten per cent of the gravel used in the United Kingdom is dredged from the sea bed, and a rapid and reliable means of detecting suitable gravel fields would be of considerable value. The accuracy of acoustic logs for ships that measure their speed by observing the Doppler shift of sound backscattered from the sea bed should also be improved by application of the new system of classification. □

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Ramsey's silicate core revisited

SIR — The case for believing that the Earth has an iron core was called the "classical view" by Birch¹ when, in 1952 he reviewed and rejected Ramsey's earlier argument²⁻⁴ that "the mantle and core are not chemically distinct". Briefly, Ramsey's proposal was that, because the terrestrial planets were not originally of the same composition (as reflected by lack of correspondence in relative mass and mean density), then the amount of iron in the cores of the planets must be out of correspondence, so that the iron core hypothesis must be rejected. Instead, Ramsey suggested that the core should be regarded as a metallic phase of a silicate such as olivine.

The arguments which led Birch to reject Ramsey's theory are still valid. One was that elements with a low atomic number would not reach the required density even at core pressures. On the basis of theory, he concluded "...that a compound such as Mg_2SiO_4 , of mean atomic number equal to 10, would reach a density of 10 only at a pressure ten times as great as the pressure of the core boundary, even in the fully ionized conditions".

In 1952, there were no laboratory data on iron at the conditions of the Earth's core, but, by 1960, because of advances in shock-wave physics, experimental data^{5,6} were sufficient for the subject to be examined again. In 1963, Birch⁷ reviewed the experimental evidence on shocked iron, and concluded that the sound velocity of silicates at core pressure could not possibly be as low as the sound velocities of the core reported by seismologists. Thus, experimental evidence backed up his earlier conclusions.

The shock-wave evidence accumulated since 1963 has repeatedly verified Birch's position, but this laboratory evidence is ignored when the proposal of a silicate core is revived, as it has been from time to time, most recently in *Nature*^{8,9}.

A *News and Views* article stated that R. Lyttleton is "entirely right to carry a torch for Ramsey's theory, which is in a category too neat not to be true". The view was expressed that the chief objection to Ramsey's hypothesis was that the experiments of high pressure geophysics had failed to verify Ramsey's hypothesis, but might be "irrelevant", although why these important experiments are irrelevant was not explained. It, therefore, is timely to review the most important experimental evidence in favour of the iron core and against the silicate core.

The chief objection to Ramsey's hypothesis is not that the shock-wave experiments have failed to find the postulated phase transition in olivine and similar minerals, although that is significant, but that shock-wave experiments have shown that pure iron at inner-core pressure and temperature has the mechanical properties that seismologists find for the inner core.

It is important to distinguish the prop-

erties of the inner core from those of the outer core; not only is one solid and the other liquid, but the former is virtually pure iron and the latter, iron diluted by lighter elements.

The argument assembled by Birch in 1963 has been largely overworked in the past decade. He found a correspondence between the velocity of sound found by seismologists and by shock-wave determinations.

The seismically determined shear and longitudinal sound velocity, V_p and V_s respectively, determined in the Earth at every depth, is related to the bulk modulus and density, K and ρ , by $\phi = (K/\rho)^{1/2} = (V_p^2 - 4/3 V_s^2)^{1/2}$. Now ϕ is the bulk sound velocity, also given by $(\delta P/\delta \rho)^{1/2}$, where P is pressure measured by shock waves. By measuring $(\delta P/\delta \rho)^{1/2}$ on iron at core pressures and temperatures, using shock waves, comparisons are made between the value of ρ for iron and the seismic ϕ in the core. Birch pointed out that the velocity of sound has an important relationship to density, forming a pattern that has come to be known as velocity-density systematics, and which has been widely used to estimate unknown compositions in the Earth's interior from seismological data. For metals under shock waves, the pattern relating the bulk sound velocity and density is dependent on the atomic number. The larger the atomic number, the smaller the velocity for a given density. Birch's (1963) graph, modified by additions, is reproduced as Fig. 1.

In the period, 1965-75, velocity-density studies showed that a phase transition in an oxide such as olivine increases both K and ρ , but that K increases as a power of ρ ($K \sim \rho^x$, $x = 4.5$)¹⁰⁻¹³. Thus, the ϕ and ρ curve for the high pressure phase in a silicate is merely a projection of the ϕ - ρ

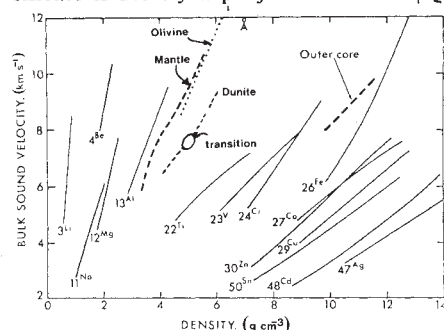


Fig. 1 Bulk sound velocity $\phi = (\delta P/\delta \rho)^{1/2}$ plotted against density ρ along the Hugoniot compression curves for metals of low atomic number⁵. The heavy dashed lines show the bulk sound velocity, $\phi = (V_p^2 - 4/3 V_s^2)^{1/2}$, of the mantle and core as determined from seismology. Compression curves are given for dunite¹⁴ through a transition at $\rho = 4.5 \text{ g cm}^{-3}$; and for dunite (A) at a very high shock pressure⁶. The ϕ versus ρ curve for shocked olivine is also shown. Silicates cluster near the mantle curve, near an effective atomic number of 14. The outer core curve is in the vicinity of metals with an effective number near 25 (modified from ref. 7).

trajectory for the low pressure phase, as shown (Fig. 1) by the dunite and olivine curves¹⁴. Because all phases of dunite lie on a line in the field of low atomic numbers, it was generally concluded that if Ramsey's theory were correct, the value of $\phi = \sqrt{K/\rho}$ of a silicate would have to be quite large at the densities found in the core, exceeding perhaps 20 km s^{-1} . In brief, the bulk sound velocity for the core is far too low to be compatible with a silicate. Whatever the core is composed of, it must have an average atomic number near 25 or 26.

Moreover, the density changes in dunite are small compared with the differences between mantle to core, as is the case for all silicates so far measured by shock-wave experimentation.

Refinements of shock-wave data on iron show that the density of the outer core is not quite as large as expected for pure iron¹⁵, so the outer core is presumed to be iron with a small concentration of solutes (as inferred by Birch⁷ from his equation of state).

Birch's 1952 and 1963 papers convinced most Earth scientists that the Earth's core is iron diluted with a small concentration of solutes. The live issue in the field became that of what those solutes could be.

Experiment after experiment has verified Birch's concept (Fig. 1). Refinements of shock-wave measurements have shown that the candidate solutes in the liquid core are S, O, or Si (refs 16-19). For example, Ahrens¹⁶ used his shock-wave experiments to demonstrate the relation of the density of Fe-S compounds to the density of the outer core as determined from seismic data. By the early 1980s, shock-wave experiments of improved resolution had shown that the data for pure iron at core conditions corresponds to the seismically determined density of the inner core^{16,19}. The Earth's inner temperatures had become better constrained by this time, so that the mechanical properties of iron (ϕ , K and ρ) could be computed; Jeanloz¹⁹ found that they agreed with the known properties of the inner core.

By 1982, the hypothesis that the inner core has the same physical properties as pure iron had been widely accepted; the question that remained was that of from which phase of pure iron it is constituted. There were two possibilities, ϵ -iron (hexagonal close-packed) or γ -iron (face-centred cubic). Several theoreticians favoured γ -iron and computed physical properties based upon this choice²⁰⁻²². But in 1980, Brown and McQueen^{23,24}, using an elegant modification of shock-wave techniques, were able to measure the longitudinal velocity, V_p , of iron at core pressures. The significance of this measurement is that, when the shock wave encounters the liquid state, V_p abruptly decreases to ϕ , since the shear velocity, V_s , vanishes, with the result that the pressure and temperature of a point on the fusion curve of iron are determined. They found²⁵ melting

at 243 ± 2 GPa and the ϵ - γ transition at the lower pressure of 200 ± 2 GPa. The corresponding temperatures are 5,000–6,000 K and $4,400 \pm 300$ K.

A simple extrapolation (from 243 to 350 GPa) of the measured V_p to inner core pressures, after correcting for the temperature expected, indicates that the V_p of pure iron at core conditions matches the seismic V_p of the inner core (Fig. 2). Since Poisson's ratio is determined by ϕ and V_p , a good match is obtained between pure iron at inner core density and that reported by seismologists for the inner core. Thus, we have agreement for the values of ϕ , α , V_p , and ρ when comparing shock-wave measurements for pure iron with those inferred by seismologists for the Earth's inner core.

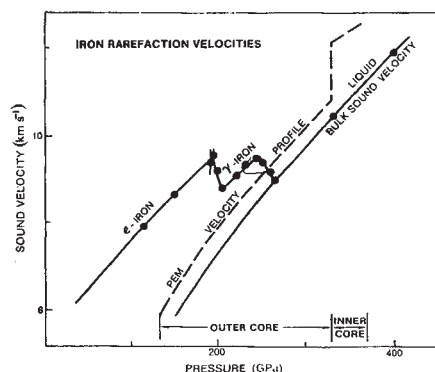


Fig. 2 The plot of compressional velocity V_p against pressure is shown by solid circles for shocked iron along the Hugoniot. At lower pressure, shocked iron is in the ϵ -phase; at 243 GPa, it transforms by a solid-solid transition to the γ -phase; and at 250 GPa, it further transforms to the liquid phase. The liquid phase is indicated by the fact that V_p has the same value as the bulk sound velocity. The dashed line shows the plot of V_p against pressure for the outer core and the inner core (called PEM), as determined from seismology³⁵. Note that V_p for shocked ϵ -iron extrapolates to higher pressure values of V_p for the inner core (modified from Fig. 1 of ref. 24).

The convergence of current research on the view that the inner core is ϵ -phase iron is supported by the extrapolation of the physical properties of the ϵ and γ phases to inner-core pressure; the ϵ -phase gives better agreement than the γ -phase²⁵. The choice of ϵ -iron is not universally accepted however; for example, Spiliopoulos and Stacey²² still hold out for γ -iron as the sole constituent of the inner core, using theoretical arguments on the behaviour of phase boundaries at high pressure.

Cosmochemists require alloys of iron for the cores of planets²⁶⁻²⁹ and many theories suggest a steady cooling of their interiors. Thus, there is fractional crystallization by a moving solid-liquid interface as the inner solid core slowly grows, tending to concentrate the solute in the outer liquid part of the core³⁰. This releases potential energy and is widely believed to be the energy source of the core dynamo that generates the Earth's magnetic field.

The shock-wave data of iron are thus

consistent with a clear model of the core: The inner core is growing, the impurities are concentrated in the outer core, the Earth is cooling and the dynamo is gravitationally driven.

The solutes in the outer core are atoms lighter than iron, according to cosmochemistry^{26,28}, so the density of the outer core is less than that of pure iron. Thermodynamically, it does not matter much what these light atoms are, for so long as the concentration is small, different solutes will equally well depress the density of pure iron to give the measured seismic velocity and density of the outer core. The detailed composition of the outer core is, of course, of intense interest to cosmochemists and cosmologists, but whether the solute is sulfur¹⁸, oxygen²⁸, silicon or hydrogen³¹, or combinations thereof, does not affect the conclusion that the Earth's outer core is predominantly iron, and that the inner core is essentially pure iron.

The details of the phase diagram of iron, up to inner-core pressures and temperatures, have been evolving for twelve years³²⁻³⁴. I accept the conclusion of Brown and McQueen that ϵ -iron constitutes the inner core^{24,25}, which means that the ϵ - γ -liquid triple point is at a pressure below that of the boundary between the inner and outer core. The phase diagram of iron (Fig. 3) is slightly adapted from ref. 34. A complete description of the phase diagram of iron at inner core conditions is presented elsewhere³⁵. There the thermodynamically derived density of iron at conditions at the top of the inner core is found to be 13.0 g cm^{-3} , compared with the seismically determined density of 12.86 g cm^{-3} by one report³⁶, and 13.4 by another report³⁷.

Thus, in the past twenty years, evidence for the iron core hypothesis has mounted to the point that it is overwhelming. Most now involved in research on the properties of the Earth's core find Ramsey's 1948 hypothesis², and Lyttleton's revival of it, somewhat quaint. To restore the acceptance of Ramsey's idea of a silicate core

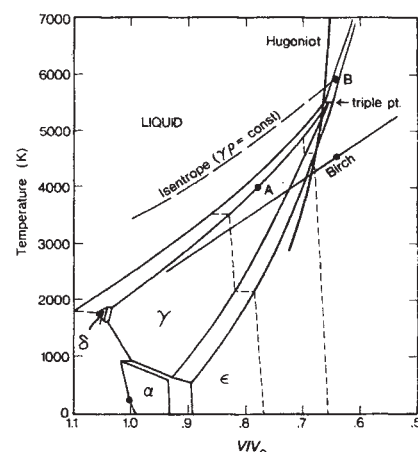


Fig. 3 The volume-temperature phase diagram of iron (adapted from Fig. 5 of ref. 34). The phase boundaries are concave upward, and connect three triple points. B marks the inner-outer core boundary pressure, and A marks the core-mantle boundary pressure. The two broken lines are isobars at 100 and 250 GPa. The upper triple point (γ - ϵ -liquid) is placed at slightly above 250 GPa. Birch's 1972 extrapolation³², based on an assumed linear T_m versus V/V_0 relationship, is shown. The temperatures along the solidus are determined by the data represented by Fig. 2.

would require not only the verified proof of the existence of a high-pressure phase change of a silicate wherein the density is doubled, but also proof that all the physical properties (K , ρ , α , V_p) of such a high pressure phase duplicate the properties of the inner core.

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- Birch, F. J. *geophys. Res.* **57**, 227-285 (1952).
- Ramsey, W.H. *Mon. Not. R. Astr. Soc.* **108**, 403-413 (1948).
- Ramsey, W.H. *Mon. Not. Roy. Astron. Soc., Geophys. Suppl.* **5**, 409-426 (1949).
- Ramsey, W.H. *Mon. Not. R. Astr. Soc., Geophys. Suppl.* **6**, 42-49 (1950).
- McQueen, R.G. & Marsh, S.P. *J. appl. Phys.* **31**, 1253-1263 (1960).
- Altschuler, L.V. & Kormer, S.B. *Bull. acad. sci., USSR, Geophys. Ser.* **1**, 18-23 (1961).
- Birch, F., in *Solids Under Pressure* (eds Paul, W. & Warschauer, D.) 137-162 (McGraw Hill, New York, 1963).
- Nature, News and Views* **300**, 681 (1982).
- Lyttleton, R.A. *Proc. R. Soc. A* **287**, 471-493 (1965).
- Anderson, O.L. *J. geophys. Res.* **71**, 4963-4971 (1966).
- Anderson, D.L. *Geophys. J. R. Astr. Soc.* **13**, 9-16 (1967).
- Shankland, T.J. *J. geophys. Res.* **77**, 3750-3760 (1972).
- Davies, G.F. *Earth Planet. Sci. Lett.* **22**, 239-41 (1974).
- McQueen, R.G., Marsh, S.P. & Fritz, J.N. *J. geophys. Res.* **72**, 4999-5036 (1967).
- Ahrens, T.J. *J. geophys. Res.* **84**, 985-998 (1979).
- Ahrens, T.J. *Science* **207**, 1035-1040 (1980).
- McQueen, R.G. & Marsh, S.P. *J. geophys. Res.* **71**, 1751-1956 (1966).
- Stevenson, D.J. *Science* **214**, 611-619 (1981).
- Jeanloz, R. *J. geophys. Res.* **84**, 6059-6069 (1979).
- Bukowinski, M. *Phys. Earth planet. Inter.* **14**, 333-343 (1977).
- Stacey, F.D. *Phys. Earth planet. Inter.* **15**, 341-348 (1977).
- Spiliopoulos, S. & Stacey, F.D. *J. Geodynamics* **1**, 61-77 (1984).
- Brown, M.J. & McQueen, R. *Geophys. Res. Lett.* **7**, 533-536 (1980).
- Brown, M.J. & McQueen, R. in *High Pressure Research in Geophysics* (eds Akimoto, A. & Manghni, M.) 611-625 (Center of Academic Publications, Tokyo, 1982); *Adv. Earth planet. Sci.* **12**, (1982).
- Brown, M.J. & McQueen, R. *J. geophys. Res.* (in the press).
- Brett, R. *Rev. Geophys. Space Phys.* **14**, 375-383 (1976).
- Murthy, V.R. & Hall, H.T. *Phys. Earth planet. Inter.* **6**, 123-130 (1972).
- Ringwood, A.E. *Geochim. J.* **11**, 111-135 (1977).
- Ito, K. *Geochim. J.* **10**, 59-64 (1976).
- Verhoogen, J. *Geophys. J. R. Astr. Soc.* **27**, 1-14 (1961); *Energetics of the Earth* (National Academy of Sciences).
- Fukei, Y., Fukuzawa, A., Watanabe, K. & Ameno, M. *J. appl. Phys.* **21**, L318-L320 (1982).
- Birch, F. *Geophys. J. R. Astr. Soc.* **29**, 373-387 (1972).
- Liu, L. *Geophys. J. R. Astr. Soc.* **43**, 697-705 (1979).
- Anderson, O.L. *Phil. Trans. R. Soc. A* **306**, 21-35 (1982).
- Anderson, O.L. *Q. J. R. Astr. Soc.* (in the press).
- Dziewonski, A.M. & Anderson, O.L. *Phys. Earth planet. Inter.* **25**, 297-356 (1981).
- Bolt, B., *Inside the Earth*, Table 4 (Freeman, San Francisco, 1982).

Unification of forces and particles in superstring theories

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Superstring field theories have emerged as potentially consistent quantum field theories that unify gravity with the other fundamental forces in an almost unique manner. They are based on the dynamics of string-like fundamental quanta rather than the point-like quanta of more familiar relativistic 'point field theories' such as Yang-Mills gauge theory or general relativity. In these theories the observed fundamental particles, such as the leptons and quarks, may arise as the ground states of a string. This amounts to a profound generalization of the conventional field theory framework.

IN this article I will review the structure of superstring theories developed over the past five years¹⁻⁴. I will describe in particular how these theories are restricted by requiring that they be consistent with quantum mechanics. Recently we have understood how these restrictions (which cannot be satisfied by point field theories) constrain the possible internal symmetry groups of superstring theories to just two possibilities, namely the special orthogonal group $SO(32)$ or the product of two exceptional groups $E_8 \times E_8$ (ref. 5). The fact that the internal symmetry is determined almost uniquely by requiring the quantum consistency of a theory containing gravity is a novel development. The hope is that this will provide a realistic unified theory that will explain experimental observations with few or no free parameters. I will outline some interesting developments in this direction.

Grand unification and supersymmetry

In the past decade a variety of Grand Unified Theories (GUTs) have been developed to unify the strong and the electro-weak interactions at an energy scale of $\sim 10^{16}$ GeV. GUTs are gauge invariant (Yang-Mills) point field theories which do not attempt to incorporate the gravitational force, and so there are few theoretical constraints on the possible symmetry groups. These groups as well as a large number of undetermined adjustable parameters are therefore chosen to account for observations as well as possible. The most favoured GUTs are based on the special unitary group $SU(5)$, the special orthogonal group $SO(10)$, or the exceptional group E_6 . In such schemes the quarks and leptons are unified into families. The quarks and leptons so far discovered make up three of these families but the question of how many families there are in total is a matter of speculation in the absence of a fundamental theory.

The observed elementary particles have masses that are tiny compared with the natural mass scale in a GUT of $\sim 10^{16}$ GeV. To a first approximation these masses are taken to be zero and the observed non-zero mass values arise from small perturbative corrections. Supersymmetry is an important ingredient in GUTs that ensures the possibility of predicting reasonable masses for these particles. It is a symmetry that relates bosons to fermions rather as internal symmetries relate groups of particles. However, as supersymmetry relates particles of different spins it is not an internal symmetry but an enlargement of the Poincaré group (the group of Lorentz transformations and translations) to the 'super-Poincaré group'. This amounts to an extension of space-time to 'superspace' that includes extra spinorial anticommuting coordinates as well as ordinary coordinates. Although theoretically appealing, the existence of supersymmetry in nature has yet to be established experimentally. It probably requires there

to be a host of particles (known as squarks, sleptons, Winos, Zinos, ...) which have not yet been discovered.

Supergravity theories are point field theories that incorporate local (that is, gauged) supersymmetry, thereby enlarging Einstein's theory of general relativity. In recent years such theories have been the focus of much research in constructing quantum theories containing gravity as well as unifying gravity with the other forces. There are fundamental problems in uniting quantum mechanics and general relativity that are associated with effects at the Planck scale (10^{-35} m). Despite early optimism that incorporating supersymmetry would resolve these problems and also unify the interactions in a geometrically appealing manner, it now seems probable that none of the supergravity theories will lead to a consistent quantum theory.

However, there is now the possibility of predicting an almost unique unified theory as a low-energy approximation to a consistent superstring theory (where the term 'low energy' means an energy low compared with the Planck scale, 10^{19} GeV).

What are superstrings?

The behaviour of a relativistic string moving in space-time differs significantly from that of a structureless point particle. Unlike a point particle, a classical relativistic string has an infinite number of vibrational modes with arbitrarily high frequencies and angular momenta. This means that in the quantum theory a single string has an infinite number of states with masses and spins which increase without limit. The scale for the mass splitting between these states is set by the string tension T (with dimension (mass)² in natural units). String theories were developed originally in the early 1970s as models for strong interaction physics (see refs 6, 7 for reviews). For example, a meson was thought of as a string with a quark attached to one end and an antiquark to the other. T was then supposed to be $\sim 1 \text{ GeV}^2$ and the excited states were interpreted as hadron resonances. The main theories of this type were the 'bosonic' string theory, which only described bosons, and the 'spinning' string theory, which incorporated fermions as well as bosons. These early string theories, however, had severe theoretical inconsistencies because the string ground state always turned out to have negative mass-squared (that is, it was a tachyon).

Superstring theories, which evolved from the spinning string theory, incorporate supersymmetry and do not suffer from the inconsistency of having tachyonic ground states. In fact, the ground states have zero mass and are simply the fundamental states of interesting supergravity point field theories. Superstring theories are therefore interpreted as theories that include gravity and the natural mass scale set by T is the Planck scale (that is, $T^{1/2} = 10^{19}$ GeV). The excited states are so massive that for many

purposes they can be taken to be infinitely heavy and the theory can be approximated by an effective point field theory of the massless states only. In other words, at energy scales well below the Planck scale (or distances $\gg 10^{-35}$ m) the string looks like a point. The quantum mechanical consistency of certain superstring field theories, however, depends crucially on the fact that at energies approaching the Planck scale the higher mass states are excited. The short distance structure therefore differs radically from that of any point field theory, such as Einstein's theory of gravity.

The classical dynamics of a relativistic string is described by an obvious generalization of the dynamics of a point particle. Just as a relativistic point particle moves along a world-line in space-time a string sweeps out a world-sheet as it moves. The obvious invariant action for a single point-particle is the length of its world-line and, similarly, the action for the simplest type of string is the area of its world-sheet⁸⁻¹⁰. For a superstring, the action is a generalization of the surface area to something like the area in superspace (which again requires additional spinorial dimensions to be adjoined to the space-time dimensions)¹¹.

The passage from classical mechanics to quantum mechanics turns out to impose severe constraints on any string theory. For example, all string theories contain massless spin-1 and spin-2 states which are associated with a Yang-Mills gauge boson and a graviton, respectively. Furthermore, the consistency of the quantum theory selects a special dimension for space-time (the 'critical' dimension). For the original (bosonic) string theory this was 26 dimensions, whereas superstring theories require 10-dimensional space-time. Just as in any theory containing gravity, the structure of space-time should be determined dynamically and so, to be of relevance to physics, the extra six dimensions must curl up and be very small (they must 'compactify'). This is analogous to the idea originally proposed by Kaluza and Klein^{12,13} in the early days of general relativity as a way of unifying gravity with electromagnetism which has received renewed interest recently in the study of supergravity point field theories. However, in the context of any point field theory, the extra dimensions make the problems of the quantum infinities of the theory even worse than they already are in four dimensions. One of the remarkable aspects of the quantum superstring field theories is that certain of them seem to have no infinities at all, even though they are defined in 10 dimensions.

Restrictions on chiral theories

A very important physical constraint on any theory is that it must give rise to the observed 'chirality' of our (approximately) four-dimensional world, that is, the lack of invariance of the laws of physics under parity inversion. In the past few years it has become clear that obtaining a chiral four-dimensional world from a higher dimensional theory imposes severe restrictions on such theories^{14,15}. It seems to be essential that the theory have chiral fermions in the higher dimensions (that is, in 10 dimensions for superstring theories) as well as a Yang-Mills gauge symmetry. In that case, the chirality of the higher-dimensional fermions can be maintained when the extra dimensions compactify, if the gauge fields twist up in a topologically non-trivial manner in the internal compact space. An example of such a non-trivial field configuration would be that of a magnetic monopole. A theory formulated in an odd number of dimensions cannot be chiral and so would seem to have little prospect of predicting sensible four-dimensional physical laws.

A theory with chiral particles will have terrible problems, however. The conservation of the Yang-Mills charges as well as the energy-momentum that is built into the classical theory may be violated in the quantum theory. Theories in which there is a quantum mechanical breakdown of such sacrosanct conservation laws are said to have 'anomalies'. These anomalies allow unphysical degrees of freedom (such as the longitudinal mode of the photon) to couple to the physical ones rendering the theory inconsistent. In 10 dimensions (and more generally in $4n+2$ dimensions), parity violation is an intrinsic property of a theory with chiral fermions (or self-dual bosons) indepen-



Fig. 1 The Yang-Mills interactions of strength g . *a*, Coupling between two open strings and a third open string. (This interaction contains the usual interaction between three massless gauge bosons). *b*, The transition between an open and a closed string.

dent of the Yang-Mills group G or to which representation of G the fermion belongs. (In contrast, in four dimensions a theory only violates parity if it contains chiral fermions in a complex representation of the gauge group.) For this reason, gravitational and Yang-Mills anomalies can generally be expected in any chiral 10-dimensional gauge theory. Requiring the absence of all anomalies leads to such stringent conditions that until recently it was thought that no interesting chiral theory could satisfy them. The results I shall describe show that for just two choices for the gauge group G it is possible to construct an anomaly-free theory. This requires a very intimate relationship between Yang-Mills and gravitational interactions, which looks somewhat contrived when viewed in terms of the effective point field theory that approximates the string at large distances. However, this unification of the Yang-Mills and gravitational forces is one of the most striking aspects of string theories.

Classification of superstring theories

Type I theories describe the dynamics of open strings that have free endpoints. These strings carry quantum numbers in the n -dimensional defining representation of a classical group $G = \text{SO}(n)$ or the symplectic group $\text{USp}(n)$ at their endpoints ($\text{SU}(n)$ leads to inconsistencies in the string field theory). This is similar to the way in which the quark quantum numbers were incorporated in the original string picture of mesons. It has not been understood how to incorporate the exceptional groups in this type of string theory. Although the string is locally invariant under two supersymmetries (that is, $N=2$), the free-end boundary conditions break this down to just one (that is, $N=1$) supersymmetry. The massless open-string states are the usual states of supersymmetric Yang-Mills theory in 10 dimensions with the gauge group G . Two open strings can interact when two ends touch and join to form one open string or, conversely, one string splits into two (Fig. 1*a*). An important corollary to this is that the two endpoints of a single string can join to form a single closed string so that type I theories necessarily contain a closed string sector (Fig. 1*b*). The massless states of a closed string form a supergravity multiplet (and do not carry the Yang-Mills quantum numbers).

This means that the effective field theory that emerges as the low energy approximation to type I theories is $N=1$ Yang-Mills coupled to $N=1$ supergravity. The fact that the existence of the Yang-Mills sector requires the existence of the gravitational sector is quite unfamiliar in ordinary point field theories. This unification of the Yang-Mills and gravitational forces leads to the relationship $\kappa = \text{const.} g^2 T$ between the gauge coupling constant, g , and the gravitational coupling (Newton's constant), κ .

Because these are chiral theories with a Yang-Mills gauge group, they may be of interest in describing observed physics for the reasons mentioned earlier. I will describe later how requiring the absence of all anomalies in type I theories restricts the choice of gauge groups to only one possibility— $\text{SO}(32)$. I will also argue that, from the point of view of the low-energy point field theory, the group $E_8 \times E_8$ is also anomaly-free. This suggests that there might also be a way of incorporating it in a type I theory.

Type II theories only involve closed strings. These can have an 'orientation' associated with the fact that waves can run around the string in two possible directions. The two orientations allow for two chiral supersymmetries, so these theories are invariant under 10-dimensional ($D=10$) $N=2$ supersymmetry. In the type IIA theory the two supercharges have opposite chirality and so the theory has no net chirality and is not of much physical interest. Its low-energy effective point field theory limit is $D=10$, $N=2$ non-chiral supergravity, which is the theory that can be thought of as the trivial reduction of $D=11$ supergravity. The type IIB theory is more interesting because it has supercharges of the same chirality. Its low-energy point field theory limit is the chiral $D=10$, $N=2$ supergravity theory (which was actually constructed this way). This theory is remarkable in being chiral and yet not having any gravitational anomalies¹⁶.

Unfortunately, the type IIB theory does not have any Yang-Mills gauge invariance and so it seems unlikely that interesting chiral compactified theories of this type exist.

Recently a third kind of superstring theory has been discovered, called the Heterotic string¹⁷. It is based on closed strings only, although it carries a Yang-Mills gauge group and has $N=1$ supersymmetry. The way the gauge quantum numbers are incorporated is very different from the method used in the type I theories. Instead of the Yang-Mills charges residing at the ends of the string there is a charge density along the string. This construction is closely related to the representation theory of Kac-Moody algebras, which has been developed over the last few years by mathematicians and physicists interested in string theories. It turns out that the only Yang-Mills groups that can be incorporated in this kind of theory are $SO(32)$ and $E_8 \times E_8$. The occurrence of just these groups is related to the fact that they are associated with the only even self-dual 16-dimensional lattices. The construction of the heterotic string theory involves some aspects of the original 26-dimensional bosonic string theory with 16 of the dimensions being the maximal torus of one or other of the two groups (see also refs 27–29). This leaves 10 space-time dimensions. In the heterotic string theory, the gauge coupling constant and the gravitational coupling constant are related by $\kappa = \text{constant} \times g/(T)^{1/2}$.

Superstring field theory

In this section I will describe the formulation of a superstring field theory which provides a framework for describing the interactions between strings in the language of quantum field theory.

At present, the field theory of strings does not have formulation based on a geometrical principle. We are restricted to describing the theories in a special parametrization of the world-sheet swept out as the string moves, known as the light-cone parametrization. This is not very satisfying for a theory which includes gravity. Nevertheless, this field theory is convenient for perturbative calculations around flat space-time and illustrates many novel features of string theories. The open-string fields, $\Phi[x(\sigma), \theta(\sigma), \tilde{\theta}(\sigma)]$, and the closed-string fields, $\Psi[x(\sigma), \theta(\sigma), \tilde{\theta}(\sigma)]$, are functionals of the string configurations. In other words, they are functions of the superspace coordinates of the whole string and are thus generalizations of conventional point fields. The superspace coordinates in the light-cone parametrization are functions of the parameter σ which labels the points on a string and consist of the transverse positions $x^i(\sigma)$ (in ten dimensions there are eight dimensions transverse to the two light-cone directions $x^\pm = (x^0 \pm x^9)/\sqrt{2}$, so $i \pm 1, 2, \dots, 8$), two grassmann spinors $\theta(\sigma)$ and $\tilde{\theta}(\sigma)$ (which are the anticommuting superspace partners of x^i) and the momentum component p^+ (which is independent of σ in this parametrization). The fields Φ and Ψ can be thought of as describing an infinite set of point fields, one for each mode of excitation of the string. The zero modes are just the usual massless fields of super-Yang-Mills and supergravity, respectively. The open-string field Φ carries the internal quantum numbers of the gauge group G (either $SO(n)$ or $USp(n)$) and is therefore a matrix in the

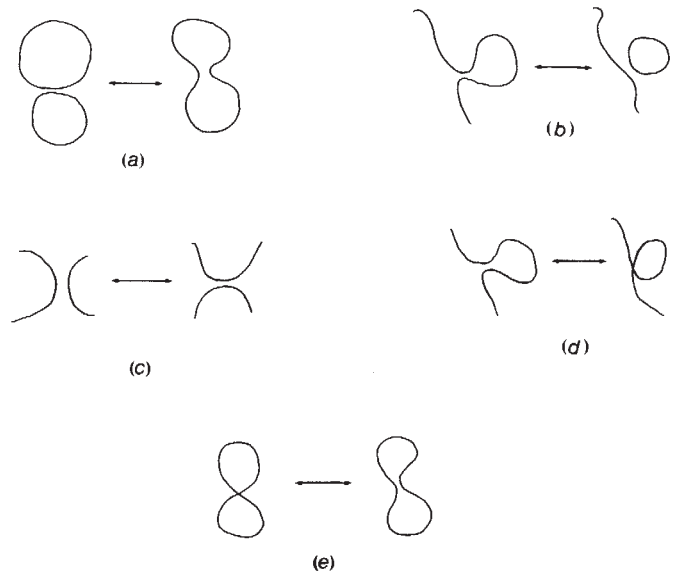


Fig. 2 The 'gravitational' interactions of strength κ . *a*, The coupling between three closed strings that contains the usual interaction between three massless gravitons. *b–e*, Other interactions that are locally the same as *a* at the point where the strings touch.

fundamental representation of the group whereas the closed-string field Ψ is a group singlet, carrying no charge.

One advantage of the light-cone parametrization is that the theory is formulated entirely in terms of a Hilbert space of physical transverse states without any redundant auxiliary or gauge degrees of freedom. However, the special parametrization destroys the manifest Poincaré invariance of the theory by picking out two directions along the light-cone in a special way. As a result, the full super-Poincaré invariance of the interacting theory is represented non-linearly on the fields. Some of the generators develop interaction terms that are cubic or of higher order in the fields. In fact, imposing the requirement that the hamiltonian of the interacting theory be invariant under super-Poincaré transformations determines it. In this manner, we have determined the superstring interaction hamiltonian which turns out to only involve local interactions only. These fall into two classes^{18,19}.

Class 1. 'Yang-Mills Interactions.' These are interactions with a coupling constant g in which two ends of a string join or, conversely, a string breaks to form two new endpoints. This gives rise to the term in the interaction hamiltonian, referred to earlier (pictured in Fig. 1*a* and cubic in the string fields); that is, it describes the breaking of one string into two or the joining of two strings into one. Exactly the same interaction is also responsible for the transition between open and closed strings pictured in Fig. 1*b* and mentioned above. The full expressions for these terms in the hamiltonian are given in ref. 18 as functional integrals over the string light-cone superspace. The fact that these two terms involve the same local interaction is very important for causality, because the two processes are indistinguishable near the joining point, differing only in the string boundary conditions far away from that point. These interactions generalize the usual cubic Yang-Mills coupling of point field theory. The zero-mode piece in the interaction of Fig. 1*a* is precisely the cubic super-Yang-Mills coupling.

Class 2. 'Gravitational Interactions.' These are interactions of strength κ in which two internal points touch and string pieces are swapped between incoming strings. An example of an interaction term of this type is shown in Fig. 2*a*, where two closed strings join to form one closed string or, conversely, one closed string splits into two. The zero-mode piece of the closed-string field is just the point superfield of supergravity and Fig. 2*a* includes the familiar coupling between three gravitons given by

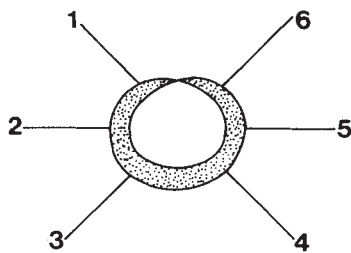


Fig. 3 The anomalous planar open-string hexagon diagram. A contribution to the anomaly in the Yang-Mills invariance.

the Einstein-Hilbert action (as well as the infinite number of other interactions involving the excited modes). For the type II theories, the interaction of Fig. 2a is the only term in the interaction hamiltonian. The type I theories, which describe unoriented strings, involve five terms with this kind of interaction, shown in Fig. 2.

The heterotic string theory describes the dynamics of an oriented closed string field. Just as with the type II theories, there is only one term in the interaction hamiltonian (Fig. 2a). This single interaction term contains both the cubic super-Yang-Mills coupling as well as the cubic supergravity coupling.

There are no higher-order contact interactions. This is quite different from point field theories like Yang-Mills or general relativity in which, for example, there are vertices which couple arbitrarily many graviton fields. These contact interactions emerge in a string theory as low-energy effective vertices resulting from the exchange of the massive string states. This is analogous to the way in which the four-fermi model of the weak interactions is now understood as a low-energy approximation to the Glashow-Weinberg-Salam theory, which involves massive W or Z boson exchange. The short-distance behaviour of superstring theories is therefore radically different from that of point field theories of gravity, as the massive modes are excited at length scales around $(T)^{-1/2}$.

These interactions, together with the free hamiltonian, lead to a set of perturbation theory rules for strings. Perturbation theory diagrams are constructed by tying together interaction vertices and free propagators to form trees and loop diagrams analogous to Feynman diagrams in point field theories.

Superstring anomaly cancellations

In earlier work we had studied one-loop perturbation theory diagrams with four external ground-state open-string particles (Yang-Mills gauge particles or their supersymmetric fermion partners). We found that, to this order, the type II theories are finite¹, unlike 10-dimensional point field theories such as type II supergravity, which diverges badly at one loop. This illustrated the possibility that superstring theory may be quantum mechanically consistent. The finiteness results from a conspiracy of divergence cancellations between the infinite numbers of supersymmetric fermion and boson states circulating around the loop. We also found that type I theories with a gauge group $G = SO(n)$ or $USp(n)$ (with general n) have a divergence (which might be eliminated by a redefinition of T). A result of the recent work to be described below is that the $SO(32)$ type I theory is finite at one loop. These properties contrast with those of supersymmetric point field theories which, though less divergent than non-supersymmetric ones, are nevertheless badly divergent in 10 dimensions.

Recently we investigated the possible anomalies that can arise in chiral string theories in a manner analogous to the occurrence of anomalies in chiral point field theories. In 10 dimensions, an anomaly in the conservation of the Yang-Mills charge is associated with a non-vanishing coupling of the longitudinal mode of a gauge particle to five physical, transverse gauge particles in a Feynman loop diagram. This hexagon diagram is the analogue of the familiar triangle diagram associated with anomalies in four-dimensional gauge theories.

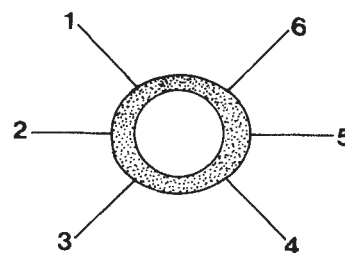


Fig. 4 One of the 32 non-orientable anomalous open-string hexagon diagrams.

Although it should be possible to calculate the anomaly in the light-cone formalism described earlier, it is easier to use a covariant formalism based on the old 'spinning string' theory. This is not initially 10-dimensionally supersymmetric, but can be made so by projection onto a suitable subspace of the full Hilbert space. The anomalous hexagon diagrams fall into three classes: (1) The first class contains only the planar diagram shown in Fig. 3. The internal propagators in the diagram are drawn as stippled strips to emphasize that they are complete string propagators, whereas the external particles are the string ground states. The diagram is therefore an annulus formed by the world-sheet of the string circulating around the loop, with all the external particles attached to one boundary. The group theory factor associated with this diagram contains a factor of n (where the group is either $SO(n)$ or $USp(n)$) which arises because the annulus in Fig. 3 has one boundary with no external particles attached. The form of the anomaly calculated from this diagram reduces at low energy (compared with $\sqrt{T}$) to the usual point-field theory hexagon diagram result, as expected.

(2) The second class of diagrams that contribute to the one-loop Yang-Mills gauge anomaly consists of those which are non-orientable, such as the example shown in Fig. 4 which has a twist in one of the string propagators. In total there are 32 diagrams of this type which have an odd number of twists and hence world-sheets which are Möbius strips. These diagrams do not have the factor on n as they only have one boundary. In other respects they each contribute to the anomaly with the same weight as the annulus in Fig. 3 but with a relative sign that is $+$ for the group $USp(n)$ and $-$ for $SO(n)$.

(3) The last class of diagrams to consider consists of the diagrams with an even (but non-zero) number of twists such as Fig. 5. Remarkably, this turns out to be free of anomalies, which is apparently at odds with the corresponding low-energy limit point-field theory hexagon diagram. The resolution of this apparent contradiction is based on the fact that the open-string hexagon diagram of Fig. 5 contains closed-string bound states in the 5-6 channel. This can be seen by distorting the stippled world-sheet of Fig. 5 to look like a cylinder, as in Fig. 6, in which case the intermediate closed-string states are apparent. As the closed-string sector includes the massless supergravity multiplet, the low energy effective point field theory must include the effects resulting from the exchange of the gravity states as well as the usual hexagon loop effects. This interplay between gravity and Yang-Mills contributions is the key to understanding how the anomalies cancel.

The total Yang-Mills gauge anomaly is therefore obtained by combining the contributions from (1) and (2). The result is proportional to $(n \pm 32)$ and hence vanishes for the group $SO(32)$. Similar arguments lead to the vanishing of gravitational anomalies (which arise from hexagon diagrams with external gravitons) for the same gauge group.

We have also reconsidered the question of infinities in scattering amplitudes. It turns out that just for the gauge group $SO(32)$ the infinities in the four-particle scattering amplitude also cancel¹⁹. In fact it is probable on very general grounds that there is a connection between the absence of anomalies in chiral string theories and the cancellation of infinities in those theories to any order in perturbation theory. This is not true in conventional point field theories.

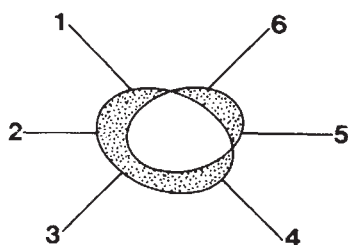


Fig. 5 A non-planar open-string hexagon diagram which turns out to be free of anomalies for any Yang-Mills gauge group.

Similar results have subsequently been found for the heterotic string theory with either gauge group $SO(32)$ or $E_8 \times E_8$.

'Low-energy' effective point theory

The fact that all anomalies cancel for specific Yang-Mills gauge groups can be explained in the language of point field theory by considering the effective theory that emerges from the string theory at low energy. In principle the superstring field theory can be formulated exactly in terms of its massless states by integrating out the massive string-field modes in the generating functional of the theory. The resulting effective point field action, which would be expressed entirely in terms of massless fields, would be horribly complicated but could be expanded in a power series in $\text{energy}/(T)^{1/2}$ for energies $< 10^{19}$ GeV. The lowest-order terms in such an expansion give the usual point-field theory action for the $D=10$ coupled $N=1$ super-Yang-Mills supergravity system that had been studied previously in this 'minimal' form^{20,21}. It turns out that to understand the absence of anomalies it is crucial to keep further terms in the effective action. Among the massless fields that occur in $D=10$, $N=1$ supergravity, there is an antisymmetric second-rank tensor, B , which plays the key role in understanding how the anomalies cancel.

The details of the anomaly cancellation in the effective point field theory are given in ref. 5 and I will simply outline the major points here. The important terms in the low-energy effective action include terms that involve the coupling of B to the other fields that are not present in the minimal action of ref. 21. These extra terms include some which spoil the Yang-Mills and the general coordinate invariances of the classical action. However, they arise in just the correct way so that they exactly compensate for the violation of these invariances by the one-loop quantum effects. The fact that this is possible depends on several remarkable identities which restrict the possible theories. For example, the cancellation of the Yang-Mills anomalies, arising from a hexagon loop with external gauge particles, restricts the possible gauge groups to ones for which

$$\text{tr } F^6 = 1/48 \text{ tr } F^2 (\text{tr } F^4 - 1/300 (\text{tr } F^2)^2)$$

where F^{ab} is an arbitrary matrix in the adjoint representation of the group and tr denotes the trace. The gravitational anomalies arise from hexagon diagrams with external gravitons and chiral fermions circulating round the loop. These fermions are the gravitino and its spin-1/2 partner as well as the spin-1/2 matter fermions from the Yang-Mills sector (of which there are m , where m is the dimension of the Yang-Mills gauge group). For the gravitational anomalies to cancel, it is necessary for the dimension of the group to be $m=496$. There are other conditions that arise by requiring the absence of anomalies which arise from hexagon diagrams with both external gravitons and Yang-Mills particles. The only groups that satisfy all these conditions are $SO(32)$ and $E_8 \times E_8$. This analysis, based entirely on an effective point field theory, suggested that as well as the $SO(32)$ superstring theory described earlier, there may be an $E_8 \times E_8$ string theory. The 'heterotic' string constructed recently¹⁷ encompasses both $SO(32)$ and $E_8 \times E_8$ and no others.

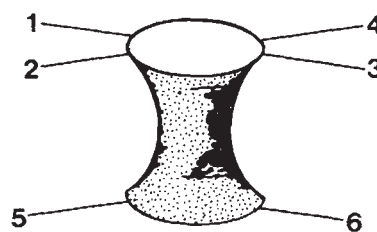


Fig. 6 Another representation for the world-sheet of the process of Fig. 5 which illustrates the closed string (gravitational) intermediate states in the channel formed by particles 5 and 6. The occurrence of gravitational states is responsible for the cancellation of the anomalies in Fig. 5.

Towards four space-time dimensions

To make contact with physics it is important to understand the predictions of superstring theories at energies well below the Planck scale. The full superstring equations should determine the structure of space-time and hopefully lead to the collapse of the extra six spatial dimensions together with a breakdown of the very large gauge group to a subgroup which might be one of the usual GUT groups or directly to the 'standard model' with symmetry $SU(3) \times SU(2) \times U(1)$. The structure of these equations is far from understood but, nevertheless, certain general features can be abstracted by studying the terms in the expansion of the low-energy effective point field theory described earlier. The equations of motion based on this low energy approximation should not be used too literally because terms that have been dropped from the expansion may be as important as the terms that have been kept. However, certain pertinent features of the effective theory are probably properties of the complete theory. For example, as emphasized in ref. 22 there is an interesting topological constraint in the theory

$$\int (\text{tr } R_{\mu\nu} R_{\rho\sigma} - 1/30 \text{tr } F_{\mu\nu} F_{\rho\sigma}) \epsilon^{\mu\nu\rho\sigma} = 0$$

where $R_{\mu\nu}^{mn}$ is the Riemann curvature tensor (which is a matrix in the $(0, 1)$ tangent-space group) and F^{ab} is the Yang-Mills strength (which is a matrix in the gauge group $SO(32)$ or $E_8 \times E_8$). The integral is over an arbitrary four-dimensional compact submanifold of the full 10-dimensional space. This is a relation between topological properties of space-time and of the Yang-Mills field configurations which connects the breaking of the Yang-Mills and the space-time symmetries. It is another aspect of the unification of Yang-Mills and gravity implied by the theory. It is also, remarkably enough, the relation that is needed to ensure that the anomaly cancellation discovered in flat 10-dimensional space-time continues to work in an arbitrary curved space-time. In general, this relation requires that F has a non-zero expectation value in some subgroup H of $SO(32)$ or $E_8 \times E_8$ in the curved dimensions, and this causes the symmetry to be broken to some commuting subgroup G which is then the symmetry group for the approximately four-dimensional theory. This may be a completely specified GUT in which the observed elementary particles are approximately massless relative to the natural mass scale of 10^{19} GeV. The spectrum of massless particles in the theory when some of the dimensions are curled up can be deduced from various index theorems on the internal space and so the number of families (and their quantum numbers) is determined by the topological structure of the theory.

One very general feature of this picture which distinguishes it from the usual Kaluza-Klein schemes is that the Yang-Mills group in the 10-dimensional theory can provide all the experimentally desirable internal symmetries. It may then be true that the dimensional compactification produces no extra gauge invariances which would happen, for example, if the internal space had no isometries. In most other schemes, these gauge symmetries are supposed to emerge from isometries of the compact internal space (for example, the $D=11$ supergravity theory has no Yang-Mills gauge group and all the observed

four-dimensional Yang-Mills symmetries are supposed to emerge from isometries of the compact seven-dimensional space in that theory). Ricci-flat spaces have no isometries and are therefore natural candidates to consider for the extra dimensions.

There has been an interesting specific suggestion as to how the observed (approximately) four-dimensional physics might emerge from the 10-dimensional theory²³. This makes use of a Ricci-flat space for the compact six dimensions. (Such Ricci-flat higher-dimensional spaces, known as 'Calabi-Yau' spaces, were conjectured to exist by Calabi and proved to exist by Yau²⁴.) Despite the fact that the full content of the string field equations is not yet understood, the authors of ref. 23 point to strong indications from the low-energy effective field theory that Ricci-flat internal manifolds are singled out. For example, if it is assumed that the four-dimensional theory has one surviving supersymmetry (desirable for phenomenological reasons), then the internal space has to be a Calabi-Yau space with holonomy group SU(3) (the curvature two-form lies in an SU(3) group), which is a severe restriction on the possible four-dimensional theories.

Furthermore, there are strong indications that the breaking of the big gauge symmetry of the 10-dimensional theory (SO(32) or $E_8 \times E_8$) is tied to the curvature in the compact dimensions. Starting with the $E_8 \times E_8$ theory in 10 dimensions, the Yang-Mills field strength associated with a SU(3) subgroup of one of the E_8 factors acquires an expectation value proportional to the curvature of the internal manifold. This causes a breaking of an SU(3) subgroup of that E_8 factor (E_8 contains $E_6 \times SU(3)$), resulting in a four-dimensional effective theory which is the supersymmetric GUT with symmetry group $E_6 \times E_8$. This is encouraging because E_6 has long been considered as a possible group for a GUT. The particle content of this unified theory is uniquely determined by specifying which Calabi-Yau space is used as the internal manifold, as the number of families is given by $1/2 \times$ the Euler characteristic of the space. For simply-connected Calabi-Yau spaces, this turns out to be a rather large number, but there are multiply-connected spaces for which the number of families is smaller. These spaces also provide an attractive topological reason for why the GUT should break down to give low-energy symmetries which might well be realistic. The extra E_8 factor describes matter which only interacts gravitationally with the charged matter in the E_6 GUT because it is neutral under the E_6 Yang-Mills group. In fact, the existence of two types of matter which only interact via the gravitational force is a general prediction of the $E_8 \times E_8$ theory when it is compactified on any internal manifold with no isometries (so that no new gauge symmetries emerge from the

compactification). This may have important implications for the 'dark matter' in the Universe²⁵ as well as for the so-called 'hidden sector' suggested by phenomenological super-unified theories for breaking the last supersymmetry.

At the present level of understanding, there is no theoretical reason for demanding one supersymmetry to survive the compactification to four dimensions. Another example mentioned as a possibility in ref. 23 is a theory in which the internal Calabi-Yau space has 0(6) holonomy and no supersymmetry survives the compactification. This results in the internal symmetry group $SO(10) \times E_8$ which is also an attractive grand unified group. These Ricci-flat internal manifolds have the virtue of not producing a cosmological constant from the compactification. But it is not yet clear why the usual purely four-dimensional sources for a cosmological constant should be absent; its absence in nature still remains a mystery.

Discussion

Recent developments have confirmed that superstring theories are interesting generalizations of point field theories containing gravity. Although these are still early days for understanding their full predictive content, the scheme proposed in ref. 23 (outlined in the previous section) suggests that there is a real chance of making contact with presently known phenomena as well as making predictions that should be testable. Other possible ways in which four-dimensional physics might emerge from these theories have been suggested in refs 23 and 26.

Our present formulation of these theories is probably not the most suitable for fully understanding their structure. For example, the relationship between the two possible Yang-Mills gauge groups and 10-dimensional space-time presumably has some geometric origin which has not been elucidated.

A more general question is why do string theories contain a massless spin-2 particle associated with gravity and a massless spin-1 particle associated with Yang-Mills theory at all? The only gauge principles used in their construction involve invariances of the two-dimensional world-sheet swept out as the string moves. String theories are not formulated in terms of any 10-dimensional gauge principle, and yet, at energy scales $< (T)^{1/2}$, they embody the principle of equivalence as well as Yang-Mills gauge invariance. Perhaps there is some generalization of the equivalence principle that gives a geometric understanding of the superstring field theory and that reduces to the usual principle at low energies. Such a generalization might be an important element in understanding the structure and hence the predictions of the theory.

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- Green, M. B. & Schwarz, J. H. *Nucl. Phys.* **B181**, 502-530 (1981); **B198**, 252-268 (1982); **B198**, 441-461 (1982); *Phys. Lett.* **109B**, 444-448 (1982).
- Green, M. B., Schwarz, J. H. & Brink, L. *Nucl. Phys.* **B198**, 474-492 (1982).
- Schwarz, J. H. *Phys. Rep.* **89**, 223-322 (1982).
- Green, M. B. *Surv. High. Energy Phys.* **3**, 127 (1983).
- Green, M. B. & Schwarz, J. H. *Phys. Lett.* **149B**, 117-122 (1984).
- Jacob, M., ed. *Dual Theory* (North-Holland, Amsterdam, 1974).
- Scherk, J. *Rev. mod. Phys.* **47**, 123 (1975).
- Nambu, Y. *Copenhagen Symp.*, unpublished (1970).
- Hara, O. *Prog. theor. Phys.* **46**, 1549 (1971).
- Goto, T. *Prog. theor. Phys.* **46**, 1560 (1971).
- Green, M. B. & Schwarz, J. H. *Phys. Lett.* **136B**, 367-370 (1984); *Nucl. Phys.* **B243**, 285-306 (1984).
- Kaluza, Th. *Sber. preuss. Akad. Wiss.* **K1**, 966 (1921).
- Klein, O. *Z. Phys.* **37**, 895 (1926).
- Witten, E., Ranjbas-Daemi, S., Salam, A. & Strathdee, J. *Nucl. Phys.* **B214**, 491 (1983).
- Witten, E. in *Proceedings of 1983 Shelter Island II Conference* (in the press).
- Alvarez-Gaume, L. & Witten, E. *Nucl. Phys.* **B234**, 269 (1984).
- Gross, D., Harvey, J., Martinec, E. & Rohm, R. *Phys. Rev. Lett.* **54**, 502-508 (1984).
- Green, M. B. & Schwarz, J. H. *Nucl. Phys.* **B243**, 475 (1984).
- Green, M. B. & Schwarz, J. H. *Phys. Lett.* **B151**, 21-25 (1985).
- Bergshoeff, M. E., B. de Wit, de Roo & van Nieuwenhuisen, P. *Nucl. Phys.* **B195**, 97-137 (1982).
- Chapline, G. & Manton, N. S. *Phys. Lett.* **120B**, 105-129 (1983).
- Witten, E. *Phys. Lett.* **149B**, 351-356 (1984).
- Candelas, P., Horowitz, G. T., Strominger, A. & Witten, E. *Nucl. Phys.* (in the press).
- Yau, S.-T. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1798 (1977).
- Kolb, B., Seckel, D. & Turner, M. *Nature* **314**, 415-419 (1985).
- Randjbar-Daemi, S., Salam, A., Sezgin, E. & Strathdee, J. *Phys. Lett.* (in the press).
- Frenkel, I. B. & Kac, V. *Inv. Math.* **62**, 23 (1980).
- Goddard, P. & Olive, D. I. *Proc. Berkeley Workshop* (in the press).
- Freund, P. G. O. *Phys. Lett.* **B151**, 387 (1985).

The shadow world of superstring theories

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Recent attempts to construct a superstring theory that unifies all the interactions of nature, including gravity, in a finite anomaly-free quantum theory have led to the speculation that there may exist another form of matter ('shadow matter') in the Universe, which only interacts with 'ordinary matter' (for example, quarks, leptons) through gravity or gravitational-strength interactions. The existence of shadow matter would have many astrophysical and cosmological implications, some of which are discussed here.

SUPERSTRING unified theories seem to offer the possibility of constructing a consistent quantum theory that unifies all interactions, including gravity¹⁻³. In fact, it has been speculated that superstrings are the only possibility for such a unification. The low-energy limit of a string theory ($E^2 \ll$ string tension $= m_{\text{pl}}^2$, where $m_{\text{pl}} = 1.22 \times 10^{19}$ GeV is the Planck mass) is an ordinary quantum field theory (QFT). Because string theories are formulated in 10 dimensions, the effective QFT we see (and feel) is the low-energy limit of a compactified string theory. Green and Schwarz⁴ have shown that the only theories that are anomaly-free and finite are those based on the gauge groups SO(32) or $E_8 \times E_8$, and have constructed a type I superstring theory based on SO(32). Recently, Gross *et al.*⁵ constructed a heterotic string theory based on either $E_8 \times E_8$ or SO(32). It has been speculated that if the gauge group is $E_8 \times E_8$, the $E_8 \leftrightarrow E_8$ symmetry may persist even in the dimensionally-reduced theory^{4,5}. If this is the case, then there would be two forms of matter: ordinary matter, whose interactions would be described by E_8 , and shadow matter; whose interactions would be described by E_8 ; these two kinds of matter would only interact through gravitational-strength interactions. We consider here some of the possible astrophysical and cosmological implications of 'generic' shadow matter; that is, matter which only interacts gravitationally with ordinary matter, and which may or may not be identical in its properties to ordinary matter. It should be stressed that although our interest in shadow matter was stimulated by recent developments in superstring theories, our discussions apply to any theory that predicts the existence of 'shadow matter' (that is, matter which only interacts gravitationally with ordinary matter). In fact, there are a variety of theories (for example, those with so-called 'hidden' sectors⁶) that predict shadow matter.

Shadow matter

We will consider several possible realizations for the shadow world. The first realization we will explore is that the shadow world exactly 'mirrors' the ordinary world. By this, we mean that both the microphysics of the shadow world (for example, symmetry breaking pattern, particle spectrum and masses) and macrophysics (for example, photon temperatures) are identical. After the Planck epoch (age of the Universe $t \leq 10^{-43}$ s, temperature of the Universe $T \geq 10^{19}$ GeV) we can be sure that the interaction between shadow and ordinary matter is unimportant on the microscopic level, because the rate for gravitational-strength interactions is much less than the expansion rate of the Universe. So although interactions among ordinary and shadow particles themselves will keep each separately in thermal equilibrium, the two worlds will not feel each other's presence on the microscopic level. If the two components are initially well mixed, they will remain well mixed until non-gravitational forces become important on macroscopic scales. In the standard cosmology, this does not occur until the later stages of galaxy formation. During the early stages of structure formation ($t \geq 10^{10}$ s, $T \leq 10$ eV), small density inhomogeneities grow via the gravitational (or Jeans) instability. When the density contrast

$\delta\rho/\rho$ begins to go non-linear (redshifts ≤ 30), non-gravitational forces begin to play an increasingly important role and these forces will act separately on ordinary and shadow matter.

Structure formation which proceeds through the fragmentation of larger objects into smaller ones through hydrodynamic or thermodynamic instabilities will lead to the segregation of ordinary and shadow matter because of the random nature of the instabilities which act independently on the two components. In the hot dark matter or 'pancake' picture⁷ large structures such as superclusters form and then fragment into galaxies through such instabilities, and so we would expect a segregation of ordinary and shadow matter on the scale of galaxies. In this case we expect to find some galaxies that are predominantly ordinary matter and others that are predominantly shadow matter, together in clusters of galaxies. If galaxies do not form from the fragmentation of a larger object, as in the cold dark matter or hierarchical picture⁸, galaxies should contain equal amounts of ordinary and shadow material. However, objects that form via instabilities within galaxies, for example, stars, will have ordinary/shadow segregation. Therefore, it is reasonable to expect that even if the disk of a galaxy contains equal amounts of ordinary and shadow matter, there may be local segregation of the two components (perhaps on scales larger than our Solar System). We note that a roughly equal component of shadow matter in the disk of our Galaxy would explain one of the several dark matter problems: that the gravitational mass of the disk (as inferred from dynamics) seems to be about twice that of the material we can see or detect (for example, stars, white dwarfs, gas, dust)⁹. One might also expect some binary systems comprised of an ordinary star and a shadow star. Such a system would manifest itself as an isolated star with a periodic proper motion. In fact there are nearby stars (at distances < 5 pc) which are suspected of having invisible companions¹⁰. Of course, there are less exotic explanations for these invisible companions; they could be neutron stars, black holes, black dwarfs or planetary-size objects.

It is possible that our Solar System formed from nebulae containing ordinary and shadow matter (although if the initial collapse of the protostellar nebulae were triggered by a shock wave in the ordinary matter, only the ordinary matter would have responded and collapsed). The shadow matter present would have formed into separate objects. However, with the possible unlikely exception of Nemesis (the death star^{11,12}), we can be confident that there are no unseen planet-sized (or larger) objects in our Solar System.

Shadow matter in the Solar System

Based on the previous discussion, it seems unlikely that the Solar System would contain a significant amount of shadow matter. Those considerations aside, what can one directly infer about the amount of shadow material in the Earth or in the Sun? Material in the Earth is supported against gravity by atomic degeneracy pressure. The same would be true of shadow material in the Earth. It would settle at the centre of the Earth (any initial

motion relative to the centre would be damped by tidal dissipation) and be distributed with an approximately constant density of the order of 10 g cm^{-3} . The mass of the Earth derived from the motion of its many satellites, $M_{\text{grav}} = 4\pi \int (\rho_s + \rho) r^2 dr = M_s + M$, and the mass derived based on seismic determinations of ρ , $M_{\text{seismic}} = 4\pi \int \rho r^2 dr = M$, are consistent at about the 10% level¹³. (Here ρ and ρ_s refer to the density of matter and shadow matter, respectively.) This means that the amount of shadow material in the Earth must be significantly less than that of ordinary material.

Next, we consider the Sun. If the amount of shadow and ordinary material were equal, then the Sun would be simultaneously a star and a shadow star, each burning its own kind of hydrogen to helium. However, as we shall see, the Sun would seem very different. If the shadow matter in the Sun were distributed identically to the ordinary matter, then the equations of stellar structure for each component are identical, and equivalent to the usual equations with G replaced by $2G_N$ ($G_N = \text{the gravitational constant, whose value is } 6.67 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1} \text{ s}^{-2}$) and the usual mass variable $M(r)$ (= mass interior to radial coordinate r) accounting for either the ordinary or shadow mass only (so that $M_{\text{total}}(r) = 2M(r)$). Using the standard $n=3$ polytrope approximation to the Sun¹⁴, it follows that the luminosity $\mathcal{L}$, rate of release of nuclear energy Q , central temperature T_c and radius R are given by

$$\begin{aligned}\mathcal{L} &\propto \mu^7 (GM)^5 G^2 T_c^{1/2} \\ Q &\propto \mu^{-3} (GM)^{-1} G^{-2} T_c^{p+3} \\ T_c &\propto \mu^{10/(p+2.5)} (GM)^{6/(p+2.5)} G^{4/(p+2.5)} \\ R &\propto \mu (GM) T_c^{-1}\end{aligned}$$

where μ is the average molecular mass per particle and $p=4$ accounts for the approximate temperature dependence of the nuclear reactions responsible for energy generation in the Sun. (Note that the central temperature is determined by the equilibrium condition: $\mathcal{L} = Q$.)

A solar mass star composed of equal quantities of ordinary and shadow matter corresponds to $G=2G_N$ and $M=M_s=M_\odot/2$. The $n=3$ polytrope model of such an object predicts: $\mathcal{L} \approx 5 \mathcal{L}_\odot$, $T_c \approx 1.5 T_{c\odot}$ and $R \approx 0.65 R_\odot$, where 'subscript zero' indicates the value of that quantity for a standard solar mass star ($M=M_\odot$, $G=G_N$) with the same chemical composition. Such a star would be easily distinguishable from 'our Sun' by its luminosity, size and solar neutrino flux (the flux of ^8B neutrinos detected by the Davis experiment is very temperature dependent, neutrino flux $\propto T_c^q$, $q \approx 13$; ref. 15). Although $\mathcal{L}$, T_c and R are all dependent on the chemical composition, it is not possible to adjust μ to obtain a model which resembles 'our Sun'. Ulrich¹⁶ and Mikkelsen and Newman¹⁷ have constructed numerical models of the Sun where they fixed GM but allowed G to differ from G_N ; from such models they concluded that G/G_N must be in the range 0.6–1.5. In passing, we note that the Chandrasekhar mass ($= M + M_s$) for a star containing equal amounts of ordinary and shadow matter is smaller than the usual value by a factor of $\sqrt{2}$.

If there is only a small amount of shadow matter in the Sun ($M_s \ll M_\odot$), the shadow material will sit at the centre of the Sun and will be supported by shadow electron degeneracy pressure. The size and central density of the shadow ball at the core of the Sun will be

$$\begin{aligned}R_s &\approx 0.2 R_\odot (M_s/M_\odot)^{1/6} \\ \rho_s &\approx 2,000 \text{ g cm}^{-3} (M_s/M_\odot)^{1/2}\end{aligned}$$

As the shadow matter would be concentrated in the core of the Sun ($r \lesssim 0.1 R_\odot$), it seems more reasonable to take GM fixed and $G \rightarrow G_N(1 + \rho_s/\rho_c)$, rather than $G \rightarrow G_N(1 + M_s/M_\odot)$, to estimate the effect of the shadow matter on the central temperature, etc. Using the $n=3$ polytrope model once again, it follows that the central temperature rises by an amount $\delta T_c/T_c \approx 6(M_s/M_\odot)^{1/2}$. As mentioned earlier, the most sensitive indicator of the central temperature of the Sun is the solar flux of ^8B

neutrinos. Taking the flux to vary as T_c^{13} and insisting that the predicted rate does not triple, say, implies that $\delta T_c/T_c \lesssim 0.09$ or $M_s/M_\odot \lesssim 0.001$.

To conclude the discussion of shadow matter in the solar neighbourhood we can say that, apart from the possibility that Nemesis is a shadow object, there can be little shadow matter in the Solar System. We again emphasize that as the shadow matter and ordinary matter could easily be segregated in the Galaxy on scales the size of the Solar System, this observation does not preclude the existence of an exactly mirror shadow world.

Primordial nucleosynthesis

Let us turn to the early Universe. The yields of big bang nucleosynthesis depend sensitively on the expansion rate of the Universe one second after the bang, when the temperature of ordinary matter was $\sim 1 \text{ MeV}$ ¹⁸. The expansion rate of the Universe at nucleosynthesis is related to the total energy density, $\rho_T = \rho + \rho_s$, by

$$\dot{R}/R = [(8\pi G_N/3)(\rho + \rho_s)]^{1/2}$$

where R is the cosmic scale factor. During this early epoch the Universe is radiation-dominated, with $\rho = g_*(\pi^2/30)T^4$, where g_* counts the effective number of degrees of freedom of particles with mass less than T

$$g_* = \sum_{\text{bosons}} g_B + 7/8 \sum_{\text{fermions}} g_F$$

The addition of shadow matter results in an effective g_* at the time of nucleosynthesis

$$\begin{aligned}\rho_T &= g_{\text{eff}}(\pi^2/30)T^4 \\ g_{\text{eff}} &= g_* + g_{*s}(T_s/T)^4\end{aligned}$$

where g_{*s} counts the effective number of degrees of freedom in the shadow world and T_s is the temperature of the shadow matter, which in general need not necessarily be the same as that of the ordinary matter. Increasing the expansion rate results in the production of more ^4He ; $\Delta Y_p \approx 0.19 \log_{10}(1 + \Delta g/g_{\text{eff}})$ ¹⁸. Here, Y_p is the mass fraction of ^4He synthesized and Δg is the change in g_{eff} .

Taking into account observational data (which strongly suggest $Y_p \approx 0.25$), uncertainty in the neutron half life and uncertainty in the calculated abundances, Yang *et al.*¹⁹ conclude that unless the number of light ($\leq$ a few MeV) neutrino species $N_\nu \leq 4$, ^4He will be overproduced. In fact if Y_p were known to be ≤ 0.250 (that is, to three significant figures), $N_\nu = 4$ is not quite allowed. Note that $N_\nu = 4$ corresponds to $g_* = 12.5$.

The limit on the number of neutrinos is really a limit on the total effective number of degrees of freedom, g_{eff} . For the purposes of our discussion, we will be conservative and take the primordial nucleosynthesis limit to g_{eff} to two significant figures, $g_{\text{eff}} \leq 13.0$. For the exact mirror shadow world ($g_{*s} = g_*$ and $T_s = T$), $g_{\text{eff}} = 25(N_\nu = 4)$, $21.5(N_\nu = 3)$ or $18(N_\nu = 2)$. Thus, big-bang nucleosynthesis eliminates exact mirror symmetry, as we know that at least two of the neutrino species are light.

Macroscopic asymmetry

Having eliminated the possibility of an exact mirror shadow world, we next discuss the possibility of a macroscopic asymmetry between the two worlds with identical microphysics. First, we note that the Solar System limits discussed in the previous section still apply. However, as before, they cannot be used to set universal limits, as shadow and ordinary matter can easily be segregated on scales as large as the Solar System. We therefore return to big bang nucleosynthesis to place limits on the macroscopic asymmetry.

A quantity which specifies the macroscopic asymmetry in a very useful way is the ratio of the entropies per co-moving volume γ

$$\gamma = \frac{s_s R^3}{s R^3} = \frac{g_{*s}(T_s)}{g_*(T)} \frac{T_s^3}{T^3}$$

where s (s_s) is the ordinary (shadow) entropy density, R is the cosmic scale factor and, as before, T and T_s are the temperatures of the ordinary and shadow matter, respectively. In the absence of entropy production in either world, γ remains constant. For the scenario at hand (identical microphysics), $g_* = g_{*s}$ so that γ , the ratio of the entropy of the shadow world to our world, is just $\gamma = r^3 \equiv (T_s/T)^3$. Lacking a detailed understanding of how the compactification from $d=10$ to $d=4$ took place, we do not know what value of γ to expect, although starting with equal amounts of entropy in the two worlds ($\gamma=1$) would seem to be a plausible assumption. For $g_{*s} = g_*$, $\gamma=1$ also implies that $T_s = T$.

The primordial nucleosynthesis constraint (that is, $g_{\text{eff}} \leq 13.0$) implies that

$$\gamma = r^3 \leq \begin{cases} 0.55 & N_\nu = 2 \\ 0.31 & N_\nu = 3 \\ 0.09 & N_\nu = 4 \end{cases}$$

If we assume that the ordinary and shadow baryon-to-entropy ratios are identical (however, see below), this implies that ordinary baryons must outnumber shadow baryons by a modest amount

$$n_B/n_{BS} \geq \begin{cases} 1.8 & N_\nu = 2 \\ 2.0 & N_\nu = 3 \\ 11.0 & N_\nu = 4 \end{cases}$$

Baryogenesis

If the temperatures of the shadow and ordinary matter are not the same, as primordial nucleosynthesis suggests, the shadow baryon asymmetry may differ from the normal baryon asymmetry even though the microphysics is the same for both worlds. In baryogenesis scenarios, the baryon-to-entropy ratio, B , is a function of $K = (\Gamma_1/H)_{T=M_X}$, where Γ_1 is the microphysical interaction rate of some baryon number violating boson X , M_X is its mass and H is the expansion rate of the Universe ($= \rho_T^{1/2} m_{\text{pl}}^{-1}$; ref. 20). If the shadow and ordinary microphysics are the same and the Universe is radiation-dominated at baryogenesis ($\rho_T \propto T^4 + T_s^4$), the relevant values of K for ordinary and shadow baryogenesis (K and K_s) will be

$$K \propto \frac{m_{\text{pl}}}{M_X[1+r^4]^{1/2}}$$

$$K_s \propto \frac{m_{\text{pl}}}{M_X[1+r^{-4}]^{1/2}}$$

where, as before, $r = T_s/T$. If no entropy is created between baryogenesis and nucleosynthesis, r must be <1 and so $K_s < K$. The baryon asymmetry decreases with increasing K (ref. 20), so the ordinary baryon asymmetry must be less than or equal to the shadow baryon asymmetry. For $K \leq 1$, the baryon asymmetry is independent of K and thus would be the same for ordinary and shadow matter. For $K \geq 1$, the baryon-to-entropy ratio is roughly proportional to K^{-1} , and the ratio of shadow to ordinary baryon numbers is given by

$$B_s/B \approx [1+r^{-4}]^{1/2}/[1+r^4]^{1/2} = r^{-2}$$

The number density of shadow baryons, n_{BS} , is related to the number density of ordinary baryons n_B by

$$\frac{n_{BS}}{n_B} = \frac{B_s}{B} \frac{T_s^3}{T^3}$$

$$= r^3 \quad (K, K_s < 1)$$

$$= r \quad (K, K_s > 1)$$

As we know that $r < 1$ from nucleosynthesis, it is difficult to imagine a scenario where shadow baryons dominate ordinary baryons (by number).

Double-bubble inflation

We have shown that γ must be <1 . Even if γ were ≥ 1 initially, it is possible that it was reduced exponentially by inflation²¹, as

we will now explain. If the two worlds are microscopically identical we would expect inflation to occur in both sectors, but not necessarily simultaneously. Remember that inflation involves a random event—the nucleation of a bubble or the formation of a fluctuation region. At the beginning of inflation the Universe is in a false vacuum state for both worlds. A bubble will nucleate (or a fluctuation region will form) for one of the worlds first, say the shadow world. As that bubble grows exponentially in physical size (that is, inflates), both T and T_s decrease exponentially and γ remains constant. When the shadow vacuum energy is converted into radiation the shadow temperature will rise to T_{RH} , and γ increases dramatically. The Universe, however, is still inflating, driven by the vacuum energy of the ordinary sector. Eventually, a bubble (or fluctuation region) forms for the ordinary world within the shadow bubble. During this second phase of inflation, the new bubble grows exponentially in size, while both T and T_s decrease exponentially. When the vacuum energy of the ordinary world is converted into radiation, the temperature of the ordinary world rises to T_{RH} , a temperature which is exponentially larger than the temperature of the shadow world. Thus, γ has been reduced to an exponentially small value by 'double-bubble inflation'. If double-bubble inflation did occur, then the shadow world is exponentially uninteresting. We note that if inflation occurs in the 10-dimensional phase, or during the compactification transition, inflation and $\gamma=1$ are not necessarily incompatible.

Asymmetric microphysics

Now let us consider the possibility that the symmetry between the ordinary world and the shadow world is broken microscopically. A scenario has been discussed recently where the ten-dimensional string theory is compactified on a six-dimensional, Ricci-flat Calabi-Yau Manifold to the four-dimensional theory $E_8 \times E_6$ (ref. 22). In this scheme, E_6 , which eventually breaks down to $SU(3) \times SU(2) \times U(1)$, is the gauge group of the ordinary world and E_8 is the gauge group of the shadow world. (Here $SU(3)$ is the gauge group of the strong interactions (QCD), and $SU(2) \times U(1)$ is the gauge group of the electroweak interactions.)

Once again we will use the entropy ratio of the two worlds, γ , to quantify the macroscopic asymmetry. However, when the microphysics of the worlds is not identical, g_{*s} is not necessarily equal to g_* , and so $\gamma=1$ does not correspond necessarily to $r = T_s/T = 1$. Nevertheless, the entropy ratio is somewhat more useful than the temperature ratio in that it remains constant in the absence of entropy production, whereas the temperature ratio varies whenever g_{*s}/g_* does

$$r = (T_s/T) = \gamma^{1/3} (g_*/g_{*s})^{1/3}$$

We note that when the microphysics of the shadow world is not identical to that of the ordinary world, there are additional means (other than inflation) for changing the entropy ratio γ . They include the very out-of-equilibrium decay of a massive particle species²³⁻²⁵ or a phase transition that is only mildly inflationary (for example, an entropy increase of $<10^6$ during the $SU(2) \times U(1) \rightarrow U(1)$ transition).

In the case of asymmetric microphysics, the very stringent baryosynthesis bound can be evaded even if the two worlds have identical initial entropies. Recall that nucleosynthesis bounds g_{eff} to be ≤ 13 . If we assume that the expansion of the Universe has been isentropic since the beginning, then g_{eff} is given by

$$g_{\text{eff}} = g_* (1 + \gamma^{4/3} (g_*/g_{*s})^{1/3})_{\text{BBN}}$$

where BBN denotes the value at big-bang nucleosynthesis, that is, when the temperature of the ordinary matter is $O(1 \text{ MeV})$. The primordial nucleosynthesis constraint $g_{\text{eff}} \leq 13.0$ results in a lower bound on $(g_{*s})_{\text{BBN}}$

$$(g_{*s})_{\text{BBN}} \geq \gamma^4 \begin{cases} 103 & N_\nu = 2 \\ 1,172 & N_\nu = 3 \\ 1.95 \times 10^5 & N_\nu = 4 \end{cases}$$

If the E_8 of the shadow world remains unbroken and the representational content of the shadow world is, as has been suggested, a single $N=1$ gauge supermultiplet, then $g_{*S}=930$. Of course, if γ is <1 (macroscopic asymmetry), the constraint could also be satisfied.

If one writes this constraint in terms of the initial temperature ratio $r_i = (T_S/T)_i$ instead of the entropy ratio γ , the constraint becomes an upper bound on g_{*S}

$$(g_{*S})_i (g_{*S})_{\text{BBN}}^{-1/4} \leq (g_{*S})_i r_i^{-3} (13 - g_{* \text{BBN}})^{3/4} g_{* \text{BBN}}^{-1}$$

where i denotes the value of a quantity at the initial epoch and BBN the value at the epoch of primordial nucleosynthesis. If $(g_{*S})_i = (g_{*S})_{\text{BBN}}$, then the constraint becomes

$$(g_{*S})_{\text{BBN}} \leq (g_{*S})_i^{4/3} r_i^{-4} \begin{cases} 0.213 & N_\nu = 2 \\ 0.095 & N_\nu = 3 \\ 0.017 & N_\nu = 4 \end{cases}$$

Massive shadow states

In gauge theories, the coupling strengths of particles are not constant, but instead evolve (or 'run') with energy. The evolution of the coupling constant g with energy scale μ is determined by the β -function

$$\mu(\partial g / \partial \mu) = \beta(g)$$

where $\beta(g)$ depends on the particle content of the theory. For the group E_8 with a single adjoint supermultiplet, $\beta(g) = -45g^3/4\pi^2$. If the coupling constant is g_0 at the Planck scale, at an energy scale μ it will be given by

$$g^2(\mu) = g_0^2(1 + g_0^2 45 \ln(\mu/m_{\text{pl}})/2\pi^2)^{-1}$$

If, for instance, $g_0 = 0.3$, then at $\mu = 10^{-2} m_{\text{pl}}$ the coupling constant will be of the order of unity. When the gauge coupling reaches order unity in a non-abelian theory, the theory can become confining, that is, the only finite energy states in the theory are bound objects which are neutral under the gauge group, and non-abelian charge is 'confined' to these states. This phenomenon occurs in QCD at an energy scale $\Lambda = O(1 \text{ GeV})$: quarks become confined in colour neutral objects, hadrons. The mass scale for the bound states is the energy scale at which the gauge coupling becomes order unity. However, there can be bound states which are significantly lighter (in fact, massless) for example, Nambu-Goldstone bosons, or states which are protected from getting mass by another symmetry. The lightness of the pion (140 MeV) is understood in terms of its being a pseudo Nambu-Goldstone boson.

If the shadow group (or some non-abelian subgroup of it) remains unbroken, some coupling may become strong, say at an energy scale Λ_S , and the shadow sector may become confining. If this does occur, then the typical mass of the physical states would be Λ_S , with the possible exception of massless Nambu-Goldstone particles. In this section we will discuss the various cosmological implications of massive shadow states. We first consider the case where none of the massive states are stable, then the case where at least one massive state is stable. Although our motivation for consideration of these states is the possibility that the shadow sector is confining, our arguments do not depend on the origin of mass for the shadow states.

Consider the case where all the massive confined states can decay into shadow states of zero mass. This is possible only if all conserved quantum numbers carried by the massive states are carried by a massless state into which they can decay. If there are such massless shadow states, the typical decay width for a massive state should be $\Gamma_S \approx \Lambda_S$. If there are no such massless shadow states, the massive ones can only decay through gravitational-strength interactions to gravitons or ordinary matter. Here, we will only treat the graviton case for which the decay width is $\Gamma_S = \Lambda_S(\Lambda_S/m_{\text{pl}})^n$, where $n=4$ for a two body bound state which annihilates to gravitons.

If $\Gamma_S \approx \Lambda_S$, the massive states decay before they become dynamically important and the story is over. If, however, at least one massive state must decay by gravitational-strength

interactions to gravitons the lifetime may be sufficiently long that the massive state(s) dominates the energy density of the Universe before they decay. When they decay into gravitons, the gravitons will have an energy density $\rho_g \approx \Lambda_S T_{\text{SD}}^3$, where T_{SD}^3 is the number density of the massive shadow particles at decay. The gravitons will act as shadow matter during nucleosynthesis as they will not thermalize with ordinary matter. At the decay epoch, ordinary matter has an energy density $\rho = T_{\text{SD}}^4 r_i^{-4}$. After decay and through nucleosynthesis ρ_g/ρ will remain constant. Primordial nucleosynthesis requires that ρ_g/ρ be <0.2 (for $N_\nu=3$); using this we can obtain a bound on Λ_S . The massive particles decay when the expansion rate of the Universe (inverse of the age of the Universe) equals their decay width (inverse of the lifetime). Setting $H = \rho^{1/2} m_{\text{pl}}^{-1} \approx T_{\text{SD}}^2 r_i^{-2} m_{\text{pl}}^{-1}$ equal to $\Gamma_S \approx \Lambda_S(\Lambda_S/m_{\text{pl}})^n$, gives $\rho_g/\rho = r^4 \Lambda_S/T_{\text{SD}} = r_i^3 (\Lambda_S/m_{\text{pl}})^{(1-n)/2}$. The limit that follows from $\rho_g/\rho < 0.2$ is

$$\Lambda_S \geq (5 r_i^3)^{2/(n-1)} m_{\text{pl}}$$

$$\Lambda_S \geq 3 r_i^2 m_{\text{pl}} \quad (n=4)$$

If $r_i = O(1)$, the theory would have to confine at a scale $\Lambda_S \approx m_{\text{pl}}$ for the massive states to be able to decay early enough to avoid interfering with nucleosynthesis.

Before leaving the question of decay we would like to comment on the possibility that the massive states decay into ordinary matter. In that case, the decays can 'heat up' the ordinary matter and increase the ordinary entropy²⁵; constraints follow from nucleosynthesis²⁵ and baryogenesis^{23,24}. Such very stringent constraints have been derived for the gravitino, a particle predicted to exist in supersymmetric theories and which decays only via gravitational strength interactions^{26,27}.

Now consider the possibility that at least one massive state is stable (or very long lived). In this case the only way such particles can diminish in number is by annihilations. If there are massless shadow states into which the massive state can annihilate, the annihilation cross section will be $\sigma_A \approx \Lambda_S^{-2}$. If there are no such massless shadow states, annihilation must be via gravitational-strength interactions into gravitons (or possibly ordinary matter) with $\sigma_A \approx \Lambda_S^{-2} (\Lambda_S/m_{\text{pl}})^n$, where for two-body annihilation via graviton exchange $n=4$. If $\sigma_A \approx \Lambda_S^{-2}$, the relic abundance of massive particles that survive annihilation has been calculated by Wolfram²⁸ and Steigman²⁹. The remaining massive shadow states which escaped annihilation would today contribute a fraction of the critical density

$$\Omega_S \approx 10^{29} r_i (\Lambda_S/m_{\text{pl}})^2 \quad (\sigma_A = \Lambda_S^{-2})$$

where, because of the inherent uncertainties, we have ignored factors of g_{*S} , g_{*S} , a logarithmic correction and the dependence on the Hubble parameter. The fact that Ω_S must be less than a few implies

$$(\Lambda_S/m_{\text{pl}}) \leq 3 \times 10^{-15} r_i^{-1/2} \quad (\sigma_A = \Lambda_S^{-2})$$

If the massive states can only annihilate into gravitons, then the annihilation rate is always much less than the expansion rate and the abundance of the massive states is not significantly reduced by annihilation. In this case the present density of the massive states will be

$$\Omega_S = 10^{27} \gamma (\Lambda_S/m_{\text{pl}}); \quad (\sigma_A = \Lambda_S^{-2} (\Lambda_S/m_{\text{pl}})^4)$$

Requiring $\Omega_S \leq O(1)$ implies that

$$\Lambda_S/m_{\text{pl}} \leq 10^{-27} \gamma^{-1}$$

To summarize the results of this section, if the decay of the massive confined states cannot occur through strong shadow forces, then for these states to avoid interfering with primordial nucleosynthesis, Λ_S/m_{pl} must be $\geq 3r_i^2$. If this inequality is not satisfied, or if some quantum number forbids decay, then the massive states must diminish in number by annihilation; to avoid contributing too much mass density at the present epoch, Λ_S/m_{pl} must be $<3 \times 10^{-15} r_i^{-1/2}$ if there are massless confined states, or Λ_S/m_{pl} must be $<10^{-27} \gamma^{-1}$ if there are no massless confined states. If one of the annihilation bounds is saturated, 'shadow relics' would be the dark matter and provide $\Omega_{\text{total}} \approx 1.0$.

Conclusion

The effect of shadow matter is hard to detect in everyday life; the reader could be living in the middle of a shadow mountain or at the bottom of a shadow ocean. But it would have many effects in the early and contemporary Universe. We have shown that an exact mirror Universe is precluded by primordial

nucleosynthesis. Our constraints, however, do not eliminate the possibility that shadow matter plays an interesting role in the evolution of the Universe.

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- Schwarz, J. *Phys. Rep.* **89C**, 223–322 (1982).
- Brink, L. *Lectures on Superstrings* (NATO Adv. Study Inst. on Supersymmetry, Bonn, 1984).
- Green, M. B. *Nature* **314**, 409–414 (1985).
- Green, M. B. & Schwarz, J. H. *Phys. Lett.* **149B**, 117–122 (1984).
- Gross, D. J., Harvey, J. A., Martinec, E. & Rohm, R. *Phys. Rev. Lett.* **54**, 503–505 (1985).
- Polonyi, J. *Budapest preprint KFKI-1977-93*.
- Zel'dovich, Ya. B. & Novikov, I. *Relativistic Astrophysics* Vol. 2 (University of Chicago Press, 1983).
- Peebles, P. J. E. *The Large-Scale Structure of the Universe* (Princeton University Press, 1980).
- Bahcall, J. N. *Astrophys. J.* **286**, 169–181 (1984).
- Mihalas, D. & Binney, J. *Galactic Astronomy*, 45 (Freeman, San Francisco, 1981).
- Whitmire, D. P. & Jackson, A. A. *Nature* **308**, 713–715 (1984).
- Davis, M., Hut, P. & Muller, R. A. *Nature* **308**, 715–717 (1984).
- Dziewonski, A. M. & Anderson, D. L. *Phys. Earth planet. Interiors* **25**, 297–306 (1981).
- Clayton, D. D. *Principles of Stellar Evolution and Nucleosynthesis* (McGraw-Hill, New York, 1968).
- Bahcall, J. N., Huebner, W. F., Lubow, S. H., Parker, P. D. & Ulrich, R. K. *Rev. mod. Phys.* **54**, 767–799 (1982).
- Ulrich, R. K. *Astrophys. J.* **188**, 369–378 (1974).
- Mikkelsen, D. R. & Newman, M. J. *Phys. Rev. D* **16**, 919–926 (1977).
- Wagoner, R. V. *Astrophys. J.* **179**, 343–360 (1973).
- Yang, J., Turner, M. S., Steigman, G., Schramm, D. N. & Olive, K. A. *Astrophys. J.* **281**, 493–511 (1984).
- Kolb, E. W. & Turner, M. S. *A. Rev. nucl. part. Sci.* **33**, 645–696 (1983).
- Linde, A. *Rep. Prog. Phys.* **47**, 925–986 (1984).
- Candelas, P., Horowitz, G., Strominger, A. & Witten, E. *Vacuum Configurations for Superstrings* (preprint, 1984).
- Dicus, D. A., Kolb, E. W. & Teplitz, V. L. *Astrophys. J.* **221**, 327–341 (1978).
- Harvey, J. A., Kolb, E. W., Reiss, D. B. & Wolfram, S. *Nucl. Phys. B* **177**, 456–460 (1981).
- Scherrer, R. J. & Turner, M. S. *Phys. Rev. D* **31**, 681–688 (1985).
- Khlopov, M. Yu. & Linde, A. *Phys. Lett.* **138B**, 265–268 (1984).
- Ellis, J., Kim, J. & Nanopoulos, D. *Phys. Lett.* **145B**, 181–186 (1984).
- Wolfram, S. *Phys. Lett.* **82B**, 65–68 (1979).
- Steigman, G. A. *Rev. nucl. part. Phys.* **29**, 313–338 (1979).

Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium *Anabaena*

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Nitrogen fixation by the cyanobacterium Anabaena is carried out in heterocysts, specialized, non-dividing cells which differentiate under conditions of ammonia or nitrate deprivation. In Anabaena, heterocyst differentiation is accompanied by rearrangement of some nitrogen fixation genes. A site-specific recombination between an 11 base-pair direct repeat sequence flanking the nifK and nifD genes removes 11 kilobases of intervening DNA, resulting in juxtaposition of the two genes and an alteration of the nifD protein-coding sequence.

OVER 30 years ago, McClintock suggested that a mobile genetic element inserted next to a specific gene could alter the time at which the gene was activated during development¹. Several examples of genome rearrangements occurring either spontaneously at low frequencies or under the control of external or developmental cues have been identified subsequently, the best example of a developmentally regulated rearrangement being that of vertebrate immunoglobulin genes². Other rearrangements have been described in some detail in the lower eukaryotes trypanosomes and yeast^{3,4}.

Genome rearrangements, termed recombinational switching, have been described in three related prokaryotic systems: *Salmonella* flagellar antigen phase variation and the host range variation of bacteriophages Mu and P1 (refs 5, 6). In each of these systems, a section of DNA is inverted by site-specific recombination in short inverted repeat sequences flanking the section. The recombination is catalysed by a recombinase encoded by DNA adjacent to one of the inverted repeats. Inversion, and consequently expression of alternate genes, is controlled by factors such as the level of recombinase. Inversion does not seem to be regulated in response to environmental cues. Transposable elements of the TnA class encode an enzyme, the product of the *tnpR* gene, that resolves cointegrate structures

by deleting a section of DNA between direct repeats⁷; *tnpR* regulates its own expression but does not respond to environmental cues. The integrated form of bacteriophage λ , however, has developmentally regulated site-specific recombination⁸. When repression is lifted in response to environmental cues, the *int* and *xis* genes are transcribed. These, together with host factors, promote site-specific recombination between direct repeats in *attL* and *attR*, resulting in excision of circular λ DNA and initiation of lytic development.

We show here two developmentally regulated rearrangements of DNA adjacent to the nitrogen fixation (*nif*) genes of *Anabaena* 7120, a filamentous cyanobacterium. One rearrangement corresponds to the excision of DNA from the chromosome, resulting in the formation of an 11-kilobase (kb) circular molecule. The other, bringing DNA to a new location adjacent to a *nif* gene, could result from a deletion, inversion or transposition. These rearrangements are specific to the differentiation of heterocysts and are triggered by the environmental cue of nitrogen deprivation.

Heterocysts

Heterocysts differentiate at regular intervals (approximately every tenth cell) along filaments of *Anabaena* deprived of ammonia or nitrate⁹ and provide an anaerobic microenvironment for nitrogen fixation. The ultimate product of nitrogen

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Fig. 1 Organization of nitrogen-fixation genes in *Anabaena* vegetative cell¹¹ and heterocyst DNA. **A**, Two contiguous *Eco*RI fragments in vegetative cell DNA containing the genes *nifK*, *nifD*, *nifH* and *nifV* or *S*. **B**, In heterocysts, the DNA is rearranged such that *nifK* and *nifD* are adjacent on the chromosome; the intervening DNA is excised from the chromosome and is found as a circle. A second rearrangement places new sequences near the *V/S* gene. The sequence originally to the right of the *V/S* gene has moved to an unknown location in heterocyst DNA. The *nifK*, *nifD* and *nifH* genes have been sequenced and their coding regions are shown¹⁵⁻¹⁷. The *nifV/S* gene has not been completely characterized, but is known to be homologous to the *nifV/S* region of *Klebsiella*. The approximate location of this homology is shown by cross-hatching¹¹. *Hind*III restriction fragments described in the text are labelled. The position of 11-bp direct repeats involved in the excision of 11 kb between *nifK* and *nifD* are indicated by solid arrows. An open arrow indicates the approximate position of the breakpoint involved in the rearrangement near the *nifV/S* gene.

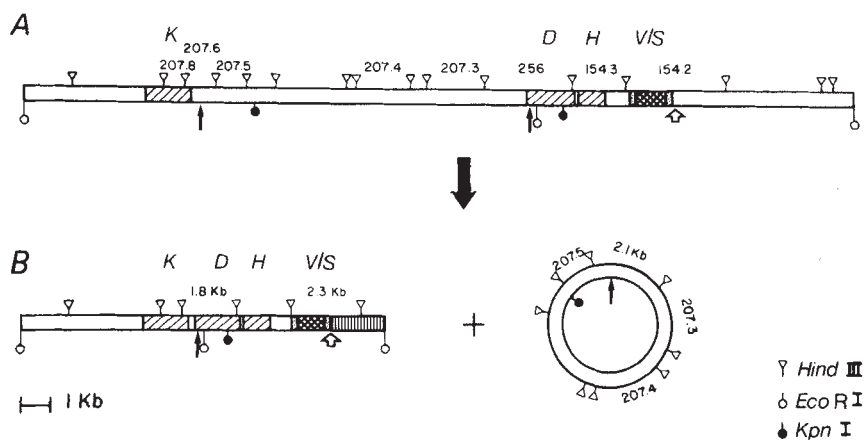
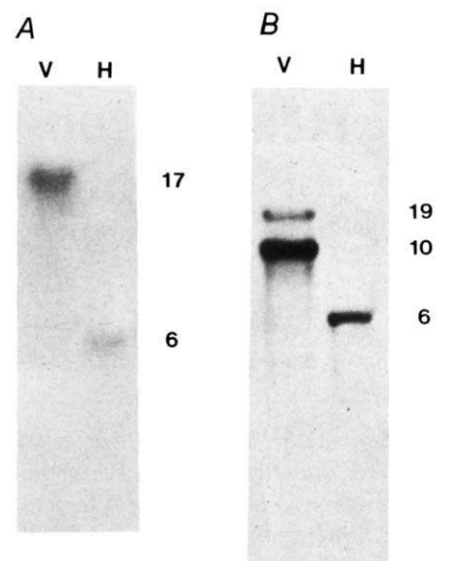


Fig. 2 Rearrangement of heterocyst DNA near the *nifK* and *nifH* genes. Vegetative cell (V) or heterocyst (H) DNA was digested with *Eco*RI and probed with **A**, 207.8 (*nifK*) or **B**, 154.3 (*nifH*). Sizes of the DNA bands are in kb.

Methods. Heterocyst purification. *Anabaena* 7120 was grown at 23–25 °C in 10 l of modified Kratz/Myers medium containing 2.5 mM (NH₄)₂SO₄ as described previously¹³. Filaments from a mid-log culture were collected and the cell pellet resuspended in 10 l of medium without (NH₄)₂SO₄ and incubated for 48–60 h. Filaments were collected by centrifugation, the cell pellets were resuspended in 100 ml of 50 mM Tris-HCl, 100 mM EDTA, pH 8 and lysozyme was added to 5 mg ml⁻¹. The filaments were incubated at room temperature for 60 min. The following procedures were then performed at 4 °C. The digestion mixture was diluted to 400 ml with 10 mM Tris-HCl, 10 mM EDTA, pH 8 (10 mM TE) and SDS was added to 0.5%. The mixture was lightly sonicated to reduce the viscosity and heterocysts were collected by centrifugation at 3,000g for 5 min. Debris was removed by resuspending the heterocysts in 10 mM TE and centrifuging at 500g for 5 min. Preparation of heterocyst DNA. Purified heterocysts were resuspended in a 25-ml Corex centrifuge tube with 5 vol. 50 mM Tris-HCl, 50 mM EDTA, pH 8.0, 0.5% Sarkosyl, and 0.5% Triton X-100 to which was added an equal volume of phenol/chloroform (1:1) and an equal volume of 0.45-mm glass beads. The tube was vortexed vigorously for 5–10 min, followed by centrifugation at 12,000g for 10 min. The aqueous supernatant was extracted twice more with phenol/chloroform (1:1), once with chloroform and then precipitated with ethanol. The nucleic acids were resuspended in 6 ml TE to which was added 3 g CsCl. This sample was layered over 2.5 ml of 5.7 M CsCl, 100 mM EDTA and centrifuged in a SW-41 rotor for 12 h at 35,000 r.p.m. RNA pellets through the lower cushion while DNA remains at the interface. The DNA was collected and banded in a CsCl-ethidium bromide gradient, dialysed, and precipitated with ethanol. Vegetative cell DNA was isolated as described previously¹¹. Restriction enzymes were used according to manufacturers' recommendations. 1 µg DNA was electrophoresed through a 0.7% agarose gel and blotted onto nitrocellulose paper. Southern blotting and hybridizations were done using standard procedures²⁵.



fixation, glutamine, is exported to neighbouring vegetative cells and, at the same time, carbohydrate is imported from vegetative cells to provide reductant for nitrogen fixation. Complete differentiation of a vegetative cell into a mature, nitrogen-fixing heterocyst requires ~30 h. Early in differentiation, unique glycolipid and polysaccharide components of the heterocyst envelope are synthesized. Proteases are induced which break down the phycobiliprotein light-collecting apparatus as well as enzymes that fix CO₂. The photosynthetic membranes are reorganized to contain photosystem I units only, thus producing ATP by cyclic photophosphorylation without the generation of O₂ associated with photosystem II. Nitrogenase, hydrogenase and enzymes of the oxidative pentose pathway are induced, together with glutamine synthetase and elements of the transport systems for glutamine and carbohydrate.

Complete differentiation of mature heterocysts is not essential for induction of nitrogenase in *Anabaena* if anaerobiosis is established in another way. This can be accomplished by removal of combined nitrogen, addition of a herbicide to inhibit O₂

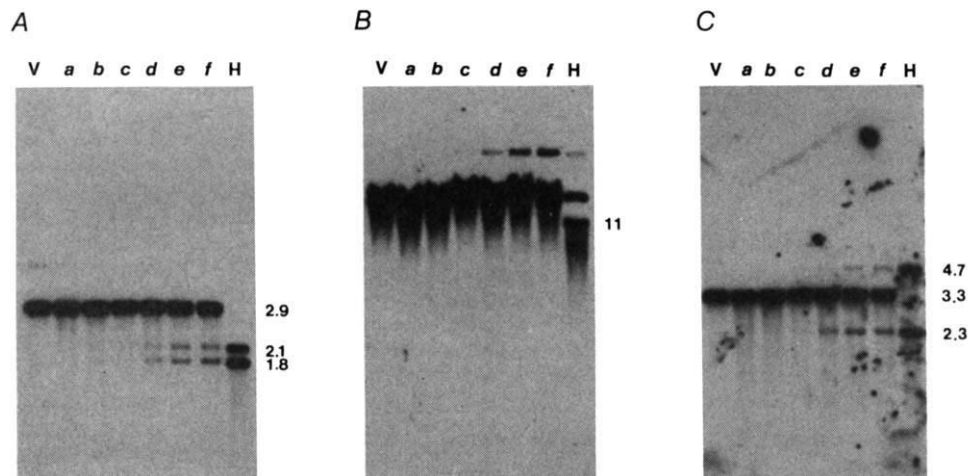
evolution by photosystem II, and bubbling the culture with argon. In these conditions, differentiation of *Anabaena* is arrested at the proheterocyst stage¹⁰⁻¹³. Admission of O₂ to such an induced culture results in rapid inactivation of nitrogenase and disappearance of *nif* gene transcripts¹².

Organization of nitrogen fixation genes

The genes encoding the polypeptide components of the nitrogenase complex (*nifH*, *D* and *K*) of *Anabaena* have been cloned from vegetative cell DNA, physically mapped and sequenced^{11,14-17}. The physical map differs from that of the corresponding genes in the well-studied bacterium *Klebsiella pneumoniae* in an unexpected way. In *Klebsiella*, these three genes are contiguous and, based on polar insertions, are co-transcribed from a promoter next to *nifH*¹⁸. In *Anabaena*, the *nifH* and *nifD* genes are contiguous, but *nifK* is separated from *nifD* by ~11 kb (Fig. 1A; ref. 11). Another region of *Anabaena* DNA identified by homology to *Klebsiella nif* gene

Fig. 3 Time course of DNA rearrangement and location of DNA breakpoints. *Hind*III-digested or undigested DNA was fractionated on 0.7% agarose gels and blotted onto nitrocellulose. **A**, *Hind*III-digested DNA probed with 256; **B**, undigested DNA probed with 207.3; **C**, *Hind*III-digested DNA probed with 154.2. (See Fig. 1 for a map of the probes.) DNA was isolated from vegetative cells (V), heterocysts (H), and at 6-h intervals during synchronous heterocyst differentiation (lanes a–f). Lanes a–f contain total DNA prepared 6, 12, 18, 24, 30 and 42 h after initiation of nitrogen starvation, respectively.

Methods. Mid-log vegetative cells from a 10-l culture containing 2.5 mM $(\text{NH}_4)_2\text{SO}_4$ were collected, washed and resuspended in 10 l of ammonia-free medium. Samples (1 l) were removed at 6-h intervals (up to 30 h) and at 42 h. The cells were collected by centrifugation, washed once with ice-cold 50 mM Tris-HCl, 50 mM EDTA, pH 8, and frozen in a dry ice ethanol bath. DNA was isolated from these cell pellets, which contained both vegetative and differentiating cells, as described in Fig. 2 legend. At 48 h, the cells in the last 4 l of culture were collected, heterocysts were isolated as described in Fig. 2 legend and DNA prepared; this DNA is shown in lanes H. Microscopic analysis showed proheterocysts at 18 h; 10% of the differentiated cells had polar bodies at 30 h.



probes is located ~1 kb to the right of *nifH* (Fig. 1A); this region contained 700 base pairs (bp) that are homologous to the *Klebsiella nifV* or *nifS* gene and has been termed *nifV/S*¹¹.

Transcription of the region of *Anabaena* DNA shown in Fig. 1A was studied by preparing RNA from cells induced for nitrogenase anaerobically. RNA from such cells hybridized with cloned DNA fragments containing *nifK*, *nifHD* and *nifV/S*, but not detectably with any of the DNA between *nifK* and *nifD*, with the possible exception of fragment 207.3 (Fig. 1A; hybridization to this fragment was about twice background¹¹). The absence of transcripts from the intervening DNA suggests that *nifK* and *nifHD* are transcribed independently under anaerobic conditions. However, we show below that late in heterocyst differentiation the heterocyst DNA is rearranged so that the *nifD* and *nifK* genes are brought together on the chromosome by excision of the intervening DNA, allowing their coordinate transcription.

Heterocyst DNA

Heterocysts can be separated from vegetative cells because of their resistance to lysozyme and physical disruption. These physical characteristics have been an obstacle to the isolation of heterocyst DNA. A procedure originally developed for the preparation of heterocyst RNA, however, was observed to yield relatively high relative molecular mass (M_r) DNA (>20 kb) from purified heterocysts. Figure 2 shows the results of hybridizing *Eco*RI-digested vegetative cell and heterocyst DNA with two probes: 207.8, entirely within *nifK*, and 154.3, containing all of *nifH* and some 5' flanking sequence (Fig. 1). The 17-kb band containing *nifK* in vegetative cell DNA is reduced to 6 kb in heterocyst DNA (Fig. 2A). Although there are other explanations, we show below that this decrease results from excision of 11 kb (see Fig. 1) from the 17-kb fragment. The probe 154.3 identifies two *Eco*RI bands in vegetative cell DNA (Fig. 2B). The 10.5-kb band contains part of *nifD* and a region homologous to the *nifV* or *nifS* genes of *Klebsiella*, as well as *nifH*¹¹. The 19-kb band contains a second region homologous to *nifH*¹¹. In heterocyst DNA, the 10.5-kb band is reduced to 6 kb (Fig. 1). The 19-kb band also appears to have disappeared, but the heterocyst DNA is sufficiently sheared before restriction endonuclease digestion such that it cannot yield 19-kb fragments with two *Eco*RI ends. Probing with the entire 19-kb *Eco*RI fragment cloned from vegetative cell DNA of other enzyme digests (for example, *Hind*III, *Cla*I) of heterocyst DNA has

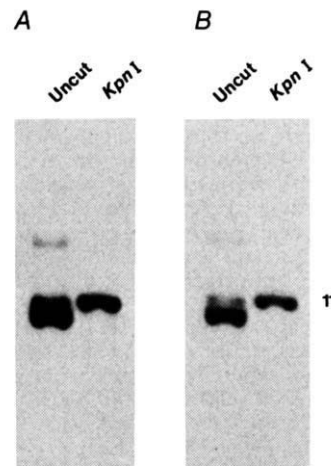


Fig. 4 Rearrangement results in the formation of an 11-kb circle. Undigested and *Kpn*I-digested heterocyst DNA was size-fractionated on a 0.7% gel, blotted onto nitrocellulose and probed with **A**, 207.4 and **B**, 207.5. Probes 207.4 and 207.5 are to the right and left, respectively, of the single *Kpn*I site found in the excised circle (Fig. 1).

Methods. Unlike the gels shown in Figs 2 and 3, the 0.7% agarose gel was run at low voltage (1.5 V cm^{-1}) in the absence of ethidium bromide for 21 h. After hybridization with probe 207.4 and exposure to film, the probe was removed by washing for 5 min in H_2O at 100 °C. Probe removal was confirmed by autoradiography and the blot was then hybridized with probe 207.5.

failed to detect evidence for rearrangement on either side of the second *nifH* gene (data not shown).

Heterocysts stain poorly with Feulgen's reagent; it has been suggested that they contain no DNA⁹. Thus it seemed possible that, during differentiation, the vegetative cell DNA was shattered in a manner analogous to that of the DNA in macronuclei of certain protozoa¹⁹. Probing with other cloned genes, however, showed no evidence of breakage in heterocyst DNA. These probes included the *Eco*RI fragment to the left of *nifK*, the *psbA* gene coding for the M_r 32,000 (32K) thylakoid membrane protein²⁰ and the *glnA* gene coding for glutamine synthetase¹³. For each probe, the pattern of hybridization with heterocyst DNA was identical to that of vegetative cell DNA (data not

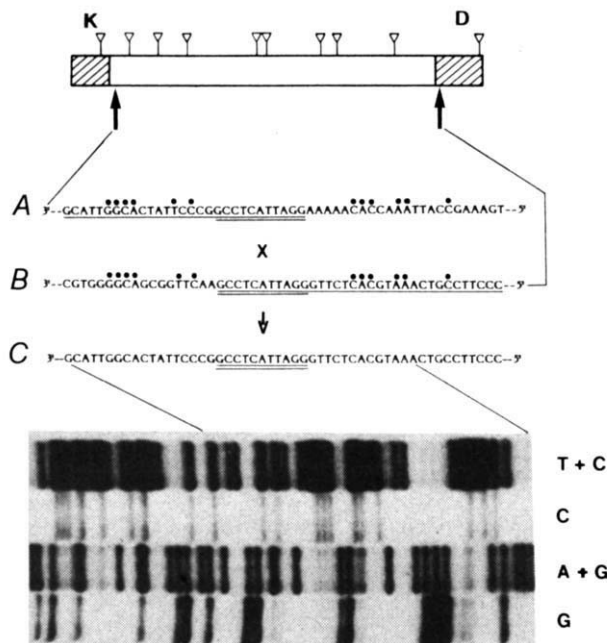


Fig. 5 DNA sequence of the fused chromosome between *nifK* and *nifD* after excision of the 11-kb fragment. The lower sequence (C) is that of the rearranged chromosome which contains one copy of the 11-bp direct repeat (double underline) flanked by sequences found 5' to the *nifK* gene and within the 3' coding region of *nifD*. The vegetative cell sequences (A, B) are shown above the rearranged sequence with the portions that are joined in the heterocyst chromosome underlined. The positions of the 11-bp direct repeats in the vegetative cell chromosome are indicated by arrows. Dots indicate partial homology in the sequences flanking the 11-bp direct repeat.

Methods. Heterocyst DNA was cloned into the vectors λ gt7ara6 and λ L47.1. DNA was partially digested with *Sau*3A and size-fractionated on a 0.8% low-gelling temperature agarose gel. Fragments between 8 and 20 kb were isolated and ligated into *Bam*HI-digested λ L47.1 arms, packaged *in vitro*, and amplified²⁵. A second library was constructed from a complete *Eco*RI digest of heterocyst DNA ligated into *Eco*RI-digested λ gt7ara6 arms. This library was screened without amplification. Several clones containing the excision were obtained from the λ L47.1 library using probes 207.6, 207.3 and 256. This library failed to yield clones of the rearranged chromosome. The λ gt7ara6 library was screened with probes 207.8 and 154.3, yielding clones of the *Eco*RI fragments containing the *nifK* and *nifH* genes, respectively. The *Eco*RI fragments were subcloned into the plasmid vector pBR328. Breakpoints were localized in the cloned DNA by probing Southern blots of restriction digests with the known breakpoint probes 256 and 207.6. Sequence analysis was by the method of Maxam and Gilbert²⁶ as modified by Smith and Calvo²⁷.

shown). The faint banding pattern seen in ethidium bromide-stained gels of restriction digests showed no apparent differences between vegetative cell and heterocyst DNA.

As the changes shown in Fig. 2 are specific for the *nif* genes, we next asked when they occur during heterocyst differentiation. For this purpose a culture was synchronously induced aerobically and samples were removed every 6 h. DNA was isolated from total cells (10% of which were differentiating), from non-induced vegetative cells and from heterocysts separated from vegetative cells (48 h after induction), as described in Fig. 2 legend. Each DNA preparation was then digested with *Hind*III (or not digested), electrophoresed and hybridized with nick-translated probes. Figure 3A shows the *Hind*III-digested DNA probed with 256, which contains the right-hand breakpoint of the excised 11-kb segment (Fig. 1). Vegetative cells show a single 2.9-kb *Hind*III band. The major band at 2.9 kb remains at all time points because of non-differentiating vegetative cells. The two new bands in heterocysts arise from the newly fused chromo-

CCC Pro	TTC Phe	CGT Arg	CAA Gln	ATG Met	CAC His	U30 TCT Ser 450	TGG Trp	GAT Asp	TAC Tyr
TCC Ser	GGC Gly	CCT Pro	TAT Tyr	CAC His	GGT Gly	TAC Tyr 460	GAC Asp	GGA Gly	TTC Phe
GAC Asp	ATG Met 470	GAT Asp	TTA Leu	GCC Ser	CTC Leu	AAC Asn	AGC Ser	GCA Pro	ACT Thr
1450 GCT Ala	CCT Pro	TGG Trp	AAG Lys	AAA Lys	CGC Ala 490	GCT Ala 490	GCA Ala	AAG Lys	GCT Ala
									1480 TTC Phe
									1490 ATT Ile
									1491 TCC Ser 497
									TAA End

Fig. 6 Sequence of the α -subunit of *Anabaena* dinitrogenase in the rearranged *nif* region of heterocysts. The underlined nucleotide sequence is the 11-bp direct repeat (5' $\rightarrow$ 3') at the site of recombination. This corresponds to nucleotides 1,353–1,363 of the published *nifD* sequence¹⁷ and to nucleotides 200–210 in the 5' region of *nifK*¹⁶. Numbers in the figure refer to the start of the *nifD* ORF¹⁷. The amino acids starting with Gly 455 replace 26 amino acids predicted from the sequence of *nifD* determined on vegetative cell DNA¹⁷. Following the TAA stop codon there are 199 bp before the ATG that starts the *nifK* ORF.

some (1.8 kb) and the joined ends of the excised circle (2.1 kb). The identity of these bands was confirmed by sequence analysis. In heterocyst DNA, the same two bands are identified by 207.6, the *Hind*III fragment containing the left-hand breakpoint 5' to the *nifK* gene (data not shown).

Figure 3B shows undigested DNA probed with 207.3, a *Hind*III fragment located entirely in the excised segment (Fig. 1). The presence of multiple discrete bands in undigested DNA from heterocysts indicates that this DNA is circular. Figure 4 shows the heterocyst DNA undigested or digested with *Kpn*I, a restriction enzyme for which there is a single site in the excised segment¹¹. The two probes used in Fig. 4 are located on either side of the *Kpn*I site (see Fig. 1); as they identify the same single band in the *Kpn*I-digested DNA, the multiple bands in undigested DNA must correspond to isomeric or polymeric forms of the same circular molecule. Different preparations of heterocyst DNA show different ratios of these various forms. For convenience, we shall refer to the 11-kb sequence as an 'excision'. The excision sequence is unique in *Anabaena* 7120 DNA (Figs 3A, 4).

The DNA samples in Fig. 3C were digested with *Hind*III and probed with 154.2, which contains the breakpoint to the right of *nifV/S* (Fig. 1). Vegetative cell DNA showed the expected single 3.3-kb band, whereas in heterocyst DNA this band was replaced by two bands of 4.7 and 2.3 kb. The new genomic location of the 4.7-kb *Hind*III fragment is unknown. The 6-kb *Eco*RI fragment identified in heterocyst DNA by the probe 154.3 (see Fig. 2) has been cloned (see Fig. 5). This clone contains the left portion of 154.2 on a 2.3-kb *Hind*III fragment, showing that this fragment in heterocyst DNA contains the *nifV/S* gene. The 6-kb *Eco*RI fragment contains, at its right end, ~2 kb of DNA originally located at least 15 kb away in vegetative cell DNA (Fig. 1B). This was determined by using the small *Hind*III/*Eco*RI fragment from the right end of the 6-kb *Eco*RI fragment (Fig. 1B) as a probe on restriction digests of vegetative cell DNA. This fragment identified a 3.5-kb *Eco*RI fragment and a ~16-kb *Hind*III fragment (data not shown). Comparison of these results with the known restriction map of the *nif* region of *Anabaena*¹¹ indicates that the new DNA originates at least 15 kb away from the *nifV/S* gene in vegetative cell DNA.

Comparison of the three parts of Fig. 3 shows that the excision and circularization of the 11-kb segment, the fusion of *nifD* and *nifK* and the rearrangement to the right of *nifV/S* occur simultaneously within the time resolution of the experiment. The rearrangements are late events in heterocyst differentiation, as is the appearance of nitrogenase activity⁹. Early proheterocysts, identified by their morphology, are first seen at 18 h, when the rearranged DNA fragments are detected faintly. At 24 h, the rearrangements are clearly visible, corresponding to the time at which *nifH* message can first be detected (data not shown).

Sequence of *nifK*/*nifD* rearrangement

To elucidate the mechanism of the excision between *nifK* and *nifD*, the two fragments of heterocyst DNA containing the breakpoints (Figs 1, 3) were cloned and sequenced. Two different clones of the excision were obtained from a partial *Sau*3A digest of heterocyst DNA cloned in the vector λ L47.1 (ref. 21). Both of these clones contained 11-kb inserts, which included the 2.1-kb *Hind*III junction fragment formed from the right portion of 207.6 and the left portion of 256 (Fig. 1). Restriction analysis showed that the two clones were circularly permuted, differing only in the *Sau*3A site originally digested. Further restriction analysis comparing the 2.1-kb junction fragment with 207.6 and 256 was used to localize the junction for sequencing.

The junction created in the chromosome by the excision was isolated from a library of *Eco*RI fragments of heterocyst DNA cloned in the vector λ gt7ara6 (ref. 22). The previously determined sequence of the junction in the excised DNA was used to predict the site of the junction on the chromosome. A portion of one sequencing gel, corresponding to the fused chromosome, is shown in Fig. 5. The new fragment (Fig. 5C) contains *nifK* 5' flanking sequence¹⁶ up to the 11-bp sequence 3'-GCCTCAT-TAGG-5' (underlined in A) and then it contains nucleotides within what was believed to be *nifD* coding sequence (underlined in B)¹⁷. The junction fragment from the excision contains the reciprocal sequence with one copy of the 11-bp sequence. Thus, the excision event is a conservative site-specific recombination between 11-bp direct repeats separated by 11 kb in the vegetative cell chromosome.

The nucleotide sequence of the *Anabaena nifD* coding region was determined previously using cloned vegetative cell DNA¹⁷. The open reading frame (ORF) predicts a polypeptide of 480 amino acids. After excision, a new ORF is created by the fusion of the *nifD* open reading frame to *nifK* 5' flanking sequence. This new ORF codes for 497 amino acids, of which the C-terminal 43 residues replace 26 C-terminal residues of the vegetative cell ORF (Fig. 6). The new ORF predicts an amino-acid sequence for the *Anabaena* nitrogenase α -subunit that is much more homologous to the *nifD* proteins of parasponia *Rhizobium* ANU289 (ref. 23) and cowpea *Rhizobium* IRC78 (ref. 24). Before the rearrangement, 1 of 26 C-terminal residues matches between *Anabaena* and *Rhizobium*; after the rearrangement, 28 of 43 residues are identical. Thus, it seems that the 11-kb element interrupts the real *nifD* coding region in *Anabaena*. The rearranged heterocyst chromosome allows the transcription of the *nifH*, *D* and *K* sequences as a polycistronic message, as in *Klebsiella*. Probes for *nifH*, *nifD* and *nifK* all identify a 4.7-kb band (with smaller transcripts) in RNA isolated from cells induced for nitrogenase, both aerobically and anaerobically (data not shown).

Why rearrange?

The two rearrangements of the *Anabaena* chromosome described here are associated with the cellular differentiation of heterocysts induced by starvation for combined nitrogen. The rearrangements are quantitative, in the sense that they occur in every genome of the differentiating cells. They are late events in the differentiation; Fig. 3 shows the first evidence for rearrangement at 18 h, while the morphological changes that characterize proheterocysts are mostly complete by 12 h. Establishment of anaerobiosis may be a necessary prerequisite for the rearrangements.

We do not yet know the functional consequence of the *nifV/S* gene rearrangement. The excision resulting in fusion of the *nifK* and *nifD* genes, however, creates an operon whose 4.7-kb transcript contains sequences of the *nifH* gene as well. It may be advantageous, and perhaps even necessary, to excise the 11-kb sequence in order to express the *nif* structural genes in *Anabaena*. The function of the 11-kb sequence in vegetative cells is unknown; it does not seem to be required for the prevention of the synthesis of nitrogenase as control of transcription initiation at the *nifH* promoter is sufficient for that purpose. We considered the possibility that a functional gene was created on the 11-kb circle by fusion of the ends of the excision, but no ORF exists in 500 bp on either side of the 11-bp repeat on the circle.

Excision of the 11-kb segment involves conservative recombination between directly repeated sequences, analogous to the excision of bacteriophage λ DNA from a lysogen⁸. Extending this analogy, it is possible that the excision is controlled by regulating synthesis of the excision enzyme. The mechanism by which the gene encoding this enzyme is regulated remains unknown, as does the regulation of transcription from the *nifH* promoter.

We are not aware of any cellular phenomena among prokaryotes or eukaryotes that display the features described here for the *Anabaena nif* gene rearrangement; that is, an environmentally triggered excision resulting in operon fusion. The closest analogy is the excision of bacteriophage λ from a lysogen. In this example, however, the elaborate controls of lysogeny have been selected to benefit the virus, rather than the host. The results presented here suggest that the 11-kb sequence confers a selective advantage on its host; how it does so remains to be discovered.

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- McClintock, B. *Cold Spring Harb. Symp. quant. Biol.* **16**, 13-47 (1951).
- Kataoka, T., Kawakami, T., Takahashi, H. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **77**, 919-923 (1980).
- Borst, P. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 622-659 (Academic, New York, 1983).
- Haber, J. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 559-619 (Academic, New York, 1983).
- Silverman, M. & Simon, M. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 537-557 (Academic, New York, 1983).
- Toussaint, A. & Resibois, A. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 105-158 (Academic, New York, 1983).
- Heffron, F. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 223-260 (Academic, New York, 1983).
- Campbell, A. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 66-103 (Academic, New York, 1983).
- Haselkorn, R. *Rev. Pl. Physiol.* **29**, 319-344 (1978).
- Rippka, R. & Stanier, R. Y. *J. gen. Microbiol.* **105**, 83-94 (1978).
- Rice, D., Mazur, B. J. & Haselkorn, R. *J. biol. Chem.* **257**, 13157-13163 (1982).

- Haselkorn, R., Rice, D., Curtis, S. E. & Robinson, S. J. *Ann. Microbiol. Inst. Pasteur* **134B**, 181-193 (1983).
- Tumer, N. E., Robinson, S. J. & Haselkorn, R. *Nature* **306**, 337-342 (1983).
- Mazur, B. J., Rice, D. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 156-160 (1980).
- Mevarech, M., Rice, D. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6476-6480 (1980).
- Mazur, B. J. & Chui, C. F. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6782-6786 (1982).
- Lammers, P. J. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4723-4727 (1983).
- Ausubel, F. M. & Cannon, F. C. *Cold Spring Harb. Symp. quant. Biol.* **45**, 487-496 (1980).
- Ammermann, D., Steinbrück, G., von Berger, L. & Henning, W. *Chromosoma* **45**, 401-429 (1974).
- Curtis, S. E. & Haselkorn, R. *Pl. molec. Biol.* **3**, 249-258 (1984).
- Loenen, W. A. M. & Brammar, W. J. *Gene* **20**, 249-259 (1980).
- Davis, R. W., Botstein, D. & Roth, J. R. *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, New York, 1980).
- Weinman, J. J., Fellows, F. F., Gresshoff, P. M., Shine, J. & Scott, K. F. *Nucleic Acids Res.* **12**, 8329-8344 (1984).
- Yun, A. C. & Szalay, A. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7358-7362 (1984).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Smith, D. R. & Calvo, J. M. *Nucleic Acids Res.* **8**, 2255-2275 (1980).

Quasar 4C39.25 is not contracting

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The milli-arc second radio structure of the quasar 4C39.25 has previously been described as consisting of two components whose angular separation remained constant at ~ 2 marc s, whereas their relative flux densities varied with time¹⁻³. This behaviour is in marked contrast to other similar sources whose radio structures expand superluminally⁴. Recently, Shaffer⁵ suggested that 4C39.25 may have been contracting superluminally in the period 1979-82. Here, based on our map of this source made from VLBI observations in 1983 at $\lambda 3.6$ cm, we conclude that this conjecture is not correct. We find three distinct components in the structure, two of which are separated by 2.0 marc s, whereas the third, presumably new and not previously reported, is situated between the other two. It is possible either that the third component is stationary and that its flux density has rapidly increased to render it visible, or that it has recently been ejected from the westernmost component.

Our VLBI observations of 4C39.25 at a radio wavelength of $\lambda 3.6$ cm took place on 10 May 1983. We recorded data for 2 min at 30 min intervals for over 7 h. We used Mark III instrumentation⁶, a synthesized bandwidth of 28 MHz, right-hand circular polarization and the following array of eight radio telescopes: 100 m, at Effelsberg, FRG; 64 m, at Robledo, Spain; 20 m, at Onsala, Sweden; 37 m, at Westford, Massachusetts, USA; 43 m, at Green Bank, West Virginia, USA; 25 m, at Fort Davis, Texas, USA; 64 m, at Goldstone, California, USA; and 40 m, at Big Pine, California, USA. Hydrogen maser frequency standards were used at each site. The data were processed at the Mark III processor of the Max-Planck-Institut für Radioastronomie in Bonn, FRG. After calibration of the visibility amplitudes⁷ we produced a map of the source using standard hybrid mapping techniques. To improve the relative calibration between the antennas we used the Cornwell and Wilkinson⁸ algorithm of self-calibration, which, given the large number of antennas available, worked very well. The resulting map is presented in Fig. 1.

We have labelled the components in Fig. 1 as *a*, *b* and *c*. The separation of the centre of component *c* from that of *a* is 2.0 ± 0.1 marc s; the separation of *b* from *a* is 1.4 ± 0.1 marc s. During the 1970s the structure was well characterized at $\lambda 2$, $\lambda 2.8$, $\lambda 3.6$ and $\lambda 6$ cm by two components separated by 2 marc s¹⁻³. More recently, Shaffer⁵ mapped the source from observations at $\lambda 2.8$ and $\lambda 3.6$ cm and found it to consist of two components, their angular separation significantly < 2.0 marc s, the western component being elongated. As a result, he conjectured that the source may be contracting, but he also recognized that motion in a source could be mimicked by the appearance of a new component. From our result we infer that the elongation of Shaffer's western component resulted from a blending of our two components *b* and *c*, and conclude that our *a* and *c* com-

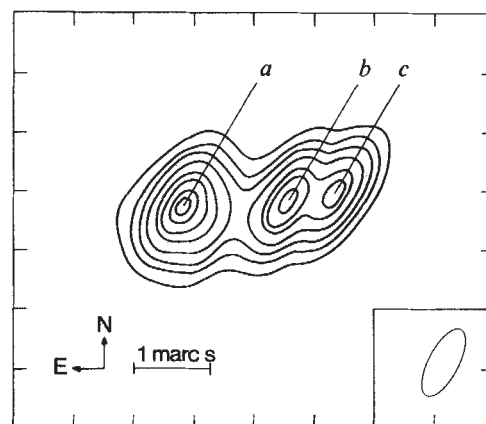


Fig. 1 Hybrid map of 4C39.25 made from $\lambda 3.6$ -cm data from epoch 10 May 1983. The restoring beam used is shown boxed in the lower right-hand-side corner. The contours specified are 4, 10, 18, 30, 50, 65, 85 and 95% of the peak brightness temperature.

ponents correspond to those observed in the 1970s, that *b* is a new component and that the source has, therefore, not contracted.

The origin of component *b* is not clear: it could be a stationary component whose flux density has rapidly increased or it may have been ejected, say, by component *c*. Curiously, according to H. D. Aller and M. F. Aller (personal communication) the flux density of 4C39.25 has been decreasing steadily at wavelengths from $\lambda 2$ to $\lambda 6$ cm since 1980 with no obvious new outbursts, whereas the linear polarization at $\lambda 2$ cm has increased from 1 to 2.5% since 1983. We cannot distinguish between the two proposed alternatives without the detailed analysis of other VLBI observations of comparable resolution made at different epochs. A detailed comparison of the results from our May 1983 observations, with observations at $\lambda 2.8$ cm from June 1982 with similar resolution (which confirm the existence of the new *b* component) and with observations at $\lambda 6$ cm from July 1982, will be presented elsewhere.

If the component *b* is stationary, 4C39.25 would surprise us again by exhibiting a milli-arc second morphology quite different from that of other radio sources whose radio spectra and variability characteristics are shared by 4C39.25. On the other hand, if component *b* has been ejected from component *c*, then its apparent motion may well be found to be superluminal. Moreover, it may overtake component *a*, as Shaffer⁵ noted. Such a possibility would be rather exciting, offering a unique opportunity to test different hypotheses on the nature of the components of compact radio sources.

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1. Shaffer, D. B. *et al. Astrophys. J.* **218**, 353-360 (1977).
2. Bååth, L. B. *et al. Astr. Astrophys.* **86**, 364-372 (1980).
3. Pauliny-Toth, I. I. K. *et al. Astr. J.* **86**, 371-385 (1981).
4. Porcas, R. *Nature* **302**, 753-754 (1983).
5. Shaffer, D. B. *Proc. IAU Symp. No. 110*, 135-136 (1984).
6. Rogers, A. E. E. *et al. Science* **219**, 51-53 (1983).
7. Marcaide, J. M. *et al. Astr. Astrophys.* **142**, 71-84 (1985).
8. Cornwell, T. J. & Wilkinson, P. N. *Mon. Not. R. astr. Soc.* **196**, 1067-1086 (1981).

The radio jet of the quasar 3C273

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Although 3C273 was one of the first quasars to be identified¹, the extended feature 3C273A, which can be detected at radio^{2,3}, optical^{4,5} and X-ray wavelengths⁶, remains an enigma. The source is an extreme example of a one-sided radio source (3C273A has no detectable counter component²) and this fact, coupled with the presence of the optical emission, makes it unlikely that 3C273A is a normal (slow-moving) radio lobe. Superluminal transverse motion^{7,8} at milliarc second scales shows that relativistic velocities occur within the quasar itself, 3C273B; it is an open question (see ref. 2) whether these velocities persist out to 3C273A. It has been widely suggested^{9,10} that Doppler beaming causes the one-sidedness of this and similar sources by suppressing the receding half of the source, but there are no spectral lines by which the Doppler shift of 3C273A could be directly measured. Thus, any (indirect) indication of the velocity is of interest. We present here new MERLIN observations of the brightness and polarization of the radio jet of 3C273 at a resolution of 0.35 arc s. One of the most marked features of our new map, the high polarization found within the head of the source, is hard to explain. If the motion is indeed fast, then relativistic aberration should be taken into account; we suggest that this leads to a natural explanation of the high observed polarization.

The MERLIN interferometer¹¹ has hitherto only been able to map brightness distributions, but we have now added the facility of mapping in linear polarization (to be described more fully elsewhere). In brief, a map of brightness is produced first, using the hybrid-mapping procedure of Cornwell and Wilkinson¹², and the relative amplitudes and phases of the polarization data¹³ are then used to construct the map of complex polarization $P = (Q + jU)$. The first polarization map, made from observations taken in April 1984, shows 3C273A at 1,666 MHz (λ 18 cm) with a resolution of 0.35 arc s (Fig. 1). The intensity data agree well with observations (A. R. Foley and R. J. Davis, personal communication) taken with MERLIN some three years earlier. Because MERLIN has few short-spacing baselines, it is difficult to map low-brightness regions at 18 cm, and neither Fig. 1 nor the earlier 18-cm map includes the fainter portions of 3C273A, which can be seen on the MERLIN map² at 73 cm, or on the Very Large Array (VLA) map³ at 6 cm. Although the core, 3C273B, does not appear on the map, it has a flux density at this epoch of 31.0 Jy and a polarization of $1.7 \pm 0.1\%$ in position angle (p.a.) $151 \pm 8^\circ$ (E-vector). These values have changed little since 1973 (R. J. Davis, personal communication).

The brightest component of 3C273A, labelled 2 in Fig. 1 (numbering from the outer end of the jet), has a linear polarization of $25 \pm 2\%$. The mean B-vector is at p.a. $128 \pm 3^\circ$, that is, 86° to the main axis of the jet, here taken as 42° . In contrast, the precursor, component 1, has a low polarization, $\approx 2 \pm 2\%$ (the uncertainty is caused by the possibility of side lobes from component 2). The magnetic field goes through a transition at or near component 3, and at all points further back than component 3 the field is parallel to the jet, to within a few degrees. Component 3 itself, which lies at an angle to the main axis, marks the start of the sinusoidal oscillation of the ridgeline (the 'wobble') noted in refs 2 and 3.

In all these respects Fig. 1 shows good agreement both with the 6-cm VLA map³ and with an unpublished VLA map at 2 cm (R. A. Perley, personal communication). It is also consistent with earlier observations¹⁴ made with single baselines at several wavelengths. The rotation measure of component 2 is $+1.80 \pm 0.20$ rad m^{-2} , and any variations over the map seem to be $\ll 0.5$ rad m^{-2} . (The value of rotation measure corresponds to 3° of rotation at λ 18 cm, which has been allowed for in determining the magnetic position angles.) We are undertaking a full comparison of all the available radio data and will submit a more

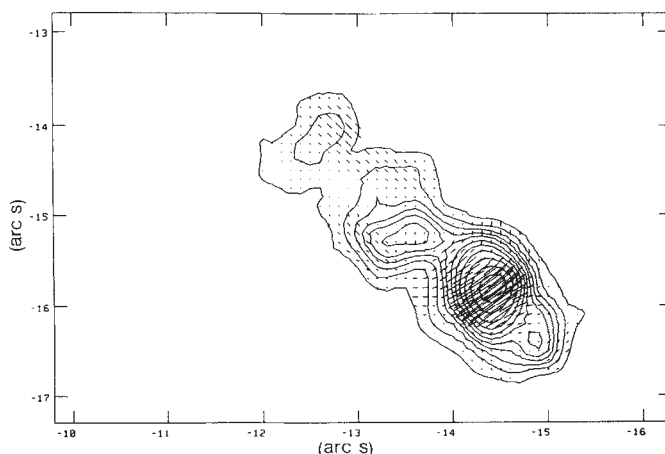


Fig. 1 MERLIN map of the radio jet in 3C273 at 1,666 MHz. The core (3C273B) is at the origin of coordinates, off the top-left of the map. The resolution is 0.35 arc s and the contours represent 0.2, 0.4, 0.6, 0.8, 1.0, 1.3, 1.6, 2.0, 2.5, 3.2, 4 and 5% of the brightness of the core, which is 31.5 Jy per beam. B-vectors of polarization (after allowing for a Faraday rotation of 3°) are superimposed on the total intensity contours. A vector 1 arc s long represents a polarized intensity of 530 mJy per beam.

detailed discussion elsewhere; our purpose here is to discuss the unexpectedly high polarization of component 2.

Laing¹⁵ has studied the polarization occurring in plasma that is sheared or compressed anisotropically. The apparent degree of polarization for a Laing sheet (that is, a thin sheet of plasma with the direction of magnetic field confined to the plane of the sheet) varies with aspect angle. If the spectral index is 0.7 as here, a polarization of 25% implies that θ_s , the angle between the line of sight and the normal to the sheet, is 47° . When the compression is partial and the magnetic field less well-ordered, as in a thick sheet, the aspect angle θ_s must be greater than 47° .

This argument seems to be completely at variance with the evidence of superluminal motion, which can only occur if the ejection axis is at a small angle to the line of sight. If Hubble's constant is $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, the observed proper motion⁷ of $0.76 \text{ marc s yr}^{-1}$ at a distance of the source ($z = 0.158$) implies a transverse velocity $\beta_{\text{obs}} = 4.6$. The most probable angle to the line of sight is $\theta_0 = \tan^{-1}(1/\beta_{\text{obs}}) = 12^\circ$, and for this angle the speed is $0.98c$, with a Lorentz factor $\gamma = 5$. A velocity β_{obs} of 4.6 cannot be observed if θ_0 is 47° .

There are several possible explanations of this apparent paradox:

(1) The superluminal motion may be entirely unconnected with the jet, but we reject this hypothesis on grounds of probability.

(2) The jet axis may be bent from $\theta = 12^\circ$ near the nucleus to 47° in the outer regions. Bends are common in jets, and a bend in position angle of 23° occurs in 3C273B^{7,16}, about 8 marc s from the core. This explanation therefore cannot be wholly excluded, but because position-angle bends are magnified by foreshortening, the real angle of bend is probably $\leq 5^\circ$, whereas a bend of at least 35° would be needed to obtain $\theta_s = 47^\circ$.

(3) Component 2 may be inclined at an angle to the axis. Hotspots set back from the head are found in other sources¹⁷ and can be interpreted as sites where the active beam intersects the side of a pre-existing cavity¹⁰. In the process, an oblique shock could be set up, which would account for component 2 if the normal to the shock were more than 35° from the jet axis. For the projection of the magnetic field to appear transverse to the axis, however, the normal to the shock must lie rather exactly in the plane of the jet axis and the line of sight. It is observed in other sources that the projected magnetic fields are invariably aligned parallel with the boundaries of emission; when seen at low resolution, the resultant is either longitudinal or transverse, rarely anything in between^{15,17}. The explanation of component 2 as an oblique shock is therefore a specially contrived geometrical solution, which could occur once, but not regularly.

Table 1 Intensities of the three components in the bright head of 3C273 corrected for Doppler beaming

Component	Distance from core (arc s)	Blue-shift	Observed flux density (mJy)	Intrinsic flux density (mJy)
1	22.1	1.2	450	216
2	21.4	3.4	1,500	13
3	20.4	4.0	450	3

We believe that other explanations that could be put forward are less probable than the following suggestion.

(4) The aspect angle may be modified by relativistic aberration. The angle θ_s , calculated above from the polarization, is that measured in the co-moving frame of the source. If component 2 is moving relativistically, aberration changes an inclination angle from θ_{jet} to θ_{obs} , given by $\sin \theta_{jet} = \eta \sin \theta_{obs}$, where η , the Doppler blue-shift, is given by $\eta = \gamma^{-1} (1 - \beta \cos \theta_{obs})^{-1}$. If we equate θ_{obs} with $\theta_0 = 12^\circ$ deduced from the superluminal motion and θ_{jet} with $\theta_s = 47^\circ$ deduced from the polarization, we obtain as the Doppler shift of component 2, $\eta \approx 3.4$. The Doppler shift measured in the frame co-moving with the quasar is larger by a factor $(1+z)_{QSO}$, that is, $\eta = 3.96$. Relative to the quasar the speed of component 2 is $\beta \approx 0.91$ and $\gamma \approx 2.4$. If component 2 is partially compressed (a thick sheet) then $\theta_s > 47^\circ$ and the values of β , γ and η given above should be regarded as lower limits.

We may apply these arguments to the precursor, component 1, which we suggest corresponds to the 'working surface' of the beam¹⁰ and moves with the speed of the bow shock. From ram pressure arguments² this speed must be sub-relativistic, in the range $0.02 < \beta < 0.3$. It follows that most of the deceleration occurs between component 2 and component 1. If the velocity of component 1 is $\beta = 0.2$, the Doppler shift $\eta = 1.22$, and the inclination angle in the emitting frame $\theta_{jet} \approx 14^\circ$. The polarization predicted for a Laing sheet would then be 2%, equal to that observed in component 1. Simulation models¹⁸ suggest that the working surface may be curved, in which case one may expect some polarization near the edge, but little in the centre of component 1. This description is entirely consistent with Fig. 1.

In this model, the relative intensities of the different components are altered by the Doppler beaming factor⁸ of $\eta^{3+\alpha}$. The Doppler shift of component 3 is not known, but the maximum value consistent with $\theta_0 = 12^\circ$ is 4.7, and if we assume a monotonic deceleration down the jet, η_3 must lie between 4.7 and $\eta_2 = 3.4$. We take $\eta_3 = 4$.

Applying a correction to the co-moving frame using Doppler shifts as above yields the intrinsic intensities given in Table 1, where it can be seen that the precursor is no longer a minor feature in front, but is intrinsically much the brightest component, as may be expected at the site of the greatest deceleration. Components 2 and 3 correspond to secondary features, which, if they were not amplified by beaming, would probably be classed as 'knots' similar to those found in many jets.

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- Schmidt, M. *Nature* **197**, 1040 (1963).
- Conway, R. G. *et al.* *Nature* **294**, 540-542 (1981).
- Perley, R. A. in *VBLI and Compact Radio Sources* (eds Fanti, R., Kellerman, K. & Setti, G.) 153-156 (Reidel, Dordrecht, 1984).
- Schmidt, G. D., Peterson, B. M. & Beaver, E. A. *Astrophys. J. Lett.* **220**, L31-L37 (1978).
- Lelievre, G. *et al.* *Astr. Astrophys.* **138**, 49-56 (1984).
- Willingale, R. *Mon. Not. R. astr. Soc.* **194**, 359-364 (1981).
- Pearson, T. J. *et al.* *Nature* **290**, 365-368 (1981).
- Blandford, R. D. & Konigl, A. *Astrophys. J.* **232**, 34-48 (1979).
- Bridle, A. H. & Perley, R. A. *Rev. Astr. Astrophys.* **22**, 319-358 (1984).
- Begelman, M. C., Blandford, R. D. & Rees, M. J. *Rev. mod. Phys.* **56**, 255-351 (1984).
- Davies, J. G., Anderson, B. & Morison, I. *Nature* **288**, 64-66 (1980).
- Cornwell, T. J. & Wilkinson, P. N. *Mon. Not. R. astr. Soc.* **196**, 1067-1086 (1981).
- Conway, R. G. & Kronberg, P. P. *Mon. Not. R. astr. Soc.* **142**, 11-32 (1969).
- Conway, R. G. & Stannard, D. *Nature* **255**, 310-312 (1975).
- Laing, R. A. *Mon. Not. astr. Soc.* **193**, 439-449 (1980).
- Davis, R. J., Stannard, D. & Conway, R. G. *Mon. Not. R. astr. Soc.* **185**, 435-440 (1978).
- Laing, R. A. *Mon. Not. astr. Soc.* **195**, 261-324 (1981).
- Wilson, M. J. & Scheuer, P. A. G. *Mon. Not. R. astr. Soc.* **205**, 449-463 (1983).

Computer classification of sea beds by sonar

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Direct sampling of the sea bed is not always feasible and can be unreliable because the samples obtained may not be representative. An alternative approach is to classify the superficial sea bed using the backscattered signal from side-scan sonars. The usual paper display contains less information than the signal creating it, because of its low dynamic range, and interpretation of sediment types is often difficult. There are also problems with statistical analysis of the signals¹. The usual methods of pattern recognition cannot resolve small-scale features in otherwise homogeneous regions² and, although the backscattering strength is a useful quantity, meaningful numerical values can be obtained only with a calibrated system³. To overcome these shortcomings we have now developed a spectral analysis method that is insensitive to system gain calibration. The method retrieves information from the signals that is well below the usual power spectrum point, thus effectively widening the system bandwidth, and can discriminate between the signals returned from six sea-bed types.

It can be shown theoretically that the scattered sound field depends on both roughness and hardness of the sediment/water interface⁴, but analytical expressions are unsuitable for most practical applications, not so much because of their complexity but because of the difficulty in quantifying roughness (statistically random corrugations) and hardness (mixed boundary conditions) of real sea beds. Although properties of scattered sound fields corresponding to an ensemble of sea-bed patches manifest only indirectly the combined effect of roughness and hardness on the incident sound field, they are nevertheless more suitable for our present semi-empirical approach. The shape of the probability distribution (of the scattered field intensity) does relate to the sea-bed roughness⁵, but is not so sensitive to its hardness.

We now consider a side-scan sonar transmitting a rectangular pulse of length T ($\gg T_0$) at carrier frequency $\omega_0 (= 2\pi/T_0)$. Received signal amplitude is proportional to the pressure of the sound field at the transducer. The received signal is a quasi-harmonic stochastic function of time ('reverberation') given by $a(t) \cos[\omega_0 t + \psi(t)]$, where $a(t)$ and $\psi(t)$ are the amplitude and the phase of the signal respectively, which are both assumed to be slowly varying functions of time in comparison with $\cos \omega_0 t$. Our method uses $a(t)$ only and yields numerical features that are invariant under the linear transformation of $a(t)$ and hence independent of the signal d.c. level and gain.

We denote by $a_i(t)$ the signal amplitude within the i th rectangular window ($i = 1, 2, \dots, n$) of a fixed time duration T_w ($\gg T$). The minimum value of the signal amplitude is set equal to zero so that $a_i(t) \geq 0$. The windows should have no temporal overlaps and can be taken from any number of pings. We further denote by $w(t)$ the time window function (other than rectangular) such that $w(t) = 0$ for $t < T_D$ and $t > T_D + T_w$, where T_D is the initial delay (measured from the instant of transmission). The resulting windowed signal can be written as $g_i(t) = w(t)a_i(t)$.

If the signal is digitized so that N samples are taken in each window with sampling interval Δt , we have $T_w = N\Delta t$. The Nyquist frequency is equal to $\pi/\Delta t$; if ω_B is the highest angular frequency of interest, the Nyquist sampling theorem implies $\Delta t \leq \pi/\omega_B$.

Performing the Fourier transform (operator $\mathcal{F}$) on the windowed amplitude $g_i(t)$ and taking the modulus squared of the resulting complex queffrequency amplitudes, the i th power spectrum is obtained as $P_i(\omega) = |\mathcal{F}\{g_i(t)\}|^2$. By averaging n power values at each frequency, we obtain the averaged power spectrum $\langle P(\omega) \rangle = n^{-1} \sum_{i=1}^n P_i(\omega)$, where it is assumed that the system

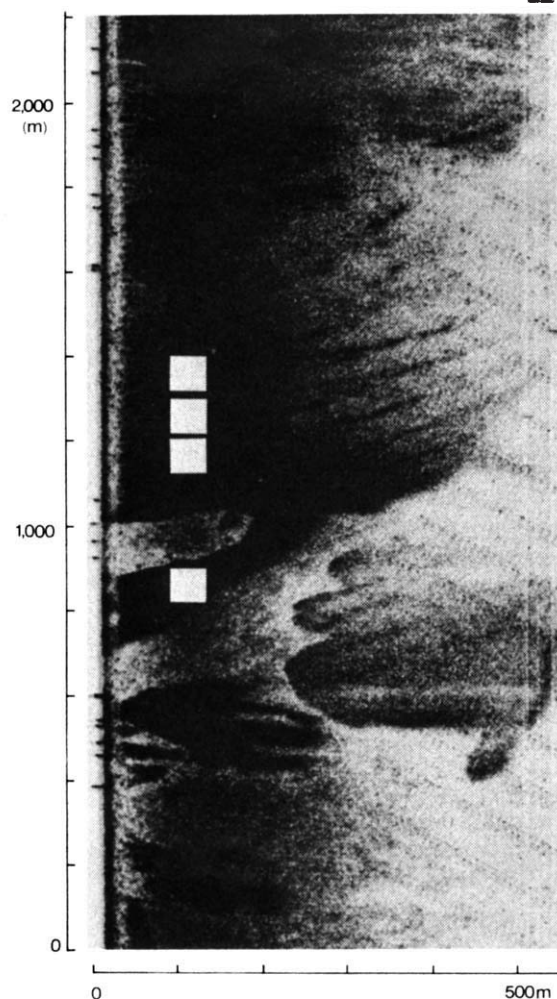


Fig. 1 Side-scan sonar paper record of sand and gravel sea-bed region. Blank squares indicate the approximate locations of four sample areas. The record width corresponds to 550 m range, the zero range being on the left-hand side. Sand and gravel appear as light and dark patches, respectively.

gain remains unaltered during the time taken to receive all n windows.

Performing the Fourier transform on the logarithm of the averaged power spectrum and taking the modulus squared of the resulting complex quantities, the power cepstrum (or simply 'cepstrum') is obtained $C(\tau) = |\mathcal{F}\{\log(P(\omega))\}|^2$, where the time-lag variable τ is called queffrequency. The cepstrum, applied to various seismic, speech analysis and machine vibration problems, appears to be more useful in certain cases^{6,7} than either the power spectrum or the auto-correlation function. However, we believe it has never been applied to side-scan sonar signals of 'reverberation' type not separating into 'echo' and 'noise'.

We define the power cepstrum integral (cumulative power cepstrum) by $I(\tau') = \int_0^{\tau'} C(\tau) d\tau / \int_0^{T_w} C(\tau) d\tau$, with the variable τ' in the range $\tau_0 \leq \tau' \leq T_w$, where $\tau_0 (\leq T)$ is a fixed interval.

We introduce two further parameters to characterize the power cepstrum integral, namely, the intercept and the slope at τ_0 given by $D_1 = 1 - I(\tau_0)$ and $D_2 = (d/d\tau')I(\tau')|_{\tau'=\tau_0}$, respectively, the cepstrum integral features, which both tend to zero when the cepstrum $C(\tau)$ contracts to the initial queffrequency range $0 \leq \tau \leq \tau_0$.

The side-scan sonar, used for data acquisition, is a standard medium range system operating at 48-kHz carrier frequency, 1-ms pulse length, 2-kHz nominal receiver bandwidth and the usual time-varied gain. The amplitude envelope is recorded on magnetic tape in analog form with 48-dB dynamic range. Hardware for data processing consists of an 8-bit A/D converter and a standard 32 K microcomputer with a dual disk drive. The amplitude envelope is digitized at a sufficiently high rate and

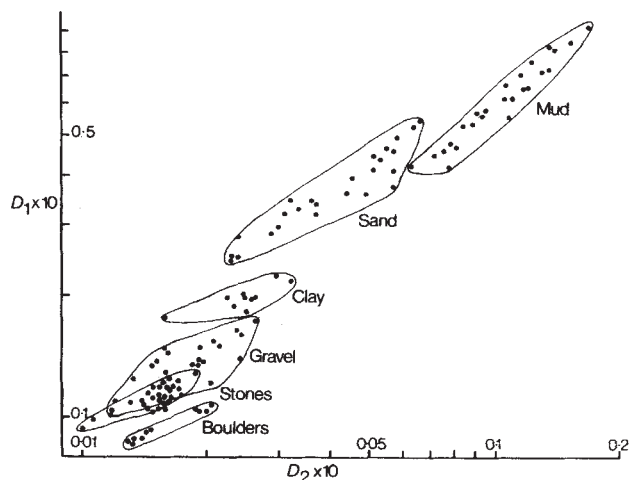


Fig. 2 Cepstrum integral features plane representation of all 120 sample areas. The boundaries to each sea-bed type are drawn by hand for clarity of display only. The time window $w(t)$ is chosen to be of the Hanning (cosine-squared) type. All Fourier transforms are performed according to the fast Fourier transform algorithm. The cepstrum is integrated numerically with a step equal to τ_0 and a straight line is interpolated through the first four points by the least-squares method in evaluating D_1 and D_2 . The gain independence of D_1 and D_2 as evaluated by our software has been tested numerically; change in gain by a factor of up to four causes deviations in D_1 and/or D_2 that are at most 5%. The numerical values of D_1 and D_2 for a given sea-bed type depend on the number of windows n , especially when n is small; both sequences (for D_1 and D_2) that correspond to $n = 1, 2, 4, 8, 16, 32$ converge relatively fast so that little can be gained by going to the values of $n > 32$.

integer values in the range 0–255 saved on disk for further processing. A typical side-scan sonar paper record is presented in Fig. 1.

All of our 120 sample areas are homogeneous as far as could be ascertained in real sea beds, are from regions familiar from previous surveys and are free from noise and interference. Based on records (like that in Fig. 1), as well as other knowledge of the regions, any one of the sample areas is assigned to only one of the six classes: mud, sand, clay, gravel, stones and boulders. The sample areas are geographically distributed as follows: mud (25 from the South Baltic Sea), sand (5 from Land's End, 10 from the Outer Hebrides and 10 from the western English Channel), clay (10 from the southern North Sea), gravel (10 from southern North Sea and 15 from western English Channel), stones (10 from South Baltic Sea and 15 from the Outer Hebrides) and boulders (10 from South Baltic Sea).

Table 1 contains the arithmetical mean and standard deviation of the two cepstrum integral features (D_1 and D_2 multiplied by 10) for the six sea-bed types. Numerical values for all 120 sample areas are presented graphically (log-log scale) in Fig 2; each sample area corresponds to a single point in the two-dimensional plot of D_1 against D_2 . Each sea-bed type corresponds to an area in (D_1, D_2) plane and the only overlap occurs between gravel and stones areas. Lower values of D_1 and D_2 generally

Table 1 Arithmetical mean and standard deviation of the cepstrum integral features for six sea-bed types

Sea-bed type	No. of sample areas	$D_1 \times 10$		$D_2 \times 10$	
		Mean	Dev.	Mean	Dev.
Mud	25	0.619	0.142	0.107	0.027
Sand	25	0.368	0.085	0.042	0.014
Clay	10	0.197	0.014	0.025	0.004
Gravel	25	0.135	0.018	0.018	0.004
Stones	25	0.111	0.007	0.015	0.002
Boulders	10	0.097	0.009	0.017	0.003

correspond to 'harder' sea-beds and the six sea-bed types span a factor of ~ 10 in the values of D_1 and D_2 .

Judging by our results, it seems certain that the cepstrum and its integral features must have an important role in the classification of sea beds. The dependence of the discriminatory ability of the method on pulse length and carrier frequency is presently being investigated especially with respect to the classification of non-homogeneous sea-bed regions (for example, sand with gravel).

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Received 11 October 1984; accepted 29 January 1985.

1. Ol'shevskii, V. V. *Characteristics of Sea Reverberation* (Consultants Bureau, New York, 1967).
2. Pace, N. G. & Dyer, C. M. *IEEE Trans. GE-17*, 52-56 (1979).
3. Wong, H.-K. & Chesterman, W. D. *J. acoust. Soc. Am.* **44**, 1713-1718 (1968).
4. Brekhovskikh, L. & Lysanov, Yu. *Fundamentals of Ocean Acoustics* (Springer, Berlin, 1982).
5. Stanton, T. K. *J. acoust. Soc. Am.* **75**, 809-818 (1984).
6. Bogert, B. P., Healey, M. J. R. & Tukey, J. W. in *Proc. Symp. Time Series Analysis* (ed. Rosenblatt, M.) 209-243 (Wiley, New York, 1963).
7. Noll, A. M. *J. acoust. Soc. Am.* **41**, 293-309 (1967).

Polarity reversal in the Solomon Islands arc

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McKenzie¹ proposed that the direction of subduction beneath an island arc will reverse following arc-continent collision. Dewey and Bird² suggested that such an arc polarity reversal occurred in northern New Guinea; Johnson and Molnar³, however, proposed two alternative models of plate convergence that could explain the observed seismicity. We present here a spatial seismicity study of the Solomon Islands region which has revealed the existence of two juxtaposed Wadati-Benioff (W-B) zones of opposite polarity. Shallow and intermediate foci define a north-east-dipping W-B zone associated with active subduction along the New Britain and San Cristobal trenches and a south-west-dipping W-B zone associated with the inactive North Solomon trench (NST). These data provide the first direct seismic evidence of a reversal in subduction polarity at an island arc.

The Solomon Islands arc forms a linear double chain of islands north-east of the New Britain and San Cristobal trenches.

The arc-trench system is part of the convergent boundary between the westward-moving Pacific Plate and the northward-moving Indo-Australian Plate (Fig. 1). Arc volcanics provide evidence of convergence between the Pacific and Indo-Australian plates in this region since the early Eocene. The polarity of the subduction is thought to have varied and a number of different models of the tectonic evolution have been proposed⁴⁻⁷. Bathymetric and seismic reflection data indicate a relict subduction zone dipping south from the North Solomon and Vitiaz trenches^{8,9}. The abnormally thick lithosphere of the Ontong Java Plateau collided with the NST sometime during the Miocene, although the exact timing remains controversial^{14,7,9}. Following the collision, the arc polarity reversed and the present regime of north-east subduction at the New Britain, San Cristobal and Vanuatu trenches began by 10 Myr BP. The hiatus in island arc volcanism lasting from late-early to early-late Miocene observed throughout the Solomon Islands^{7,10} may be evidence of a temporary cessation of subduction during the polarity reversal.

Earthquake hypocentres, volcano locations and major tectonic features of the Solomon Islands region are illustrated in Fig. 2. Hypocentres covering the period 1 January 1964 to 30 June 1984 are taken from the International Seismological Centre (ISC) catalogue and include all events with body-wave magnitude ≥ 4.7 and recorded by more than 15 stations. Three primary features are evident from Fig. 2. First, the spatial density of earthquake foci changes along strike; shallow (< 70 km) and intermediate (70-300 km) activity is concentrated in two zones of greatest density under the northwestern and southeastern parts of the arc. Second, a zone of low-density shallow seismicity is associated with the active spreading system extending east across the Woodlark Basin. The low density of seismicity in the central Solomon Islands (from 156-159°E) coincides with the site of subduction of the young Woodlark Basin lithosphere. Third, diffuse shallow and intermediate seismicity is also observed north-east of New Georgia and Guadalcanal, extending to Santa Isabel and Malaita, which provides evidence of lithosphere previously subducted at the NST.

Four representative seismicity cross-sections located in Fig. 2 and one longitudinal section are shown in Fig. 3. The top of the subducted lithosphere is assumed to extend from the topographic trench axis downward along the upper limit of the concentrated seismicity. Both the dip and the depth-extent of the north-east-dipping W-B zone associated with subduction at the New Britain and San Cristobal trenches vary significantly along strike (Figs 2, 3). North of Bougainville, Fig. 3A shows a well-defined seismic zone dipping at an initially shallow angle, then steepening rapidly until becoming almost vertical below 100 km. Teleseismic activity is virtually absent in the overriding

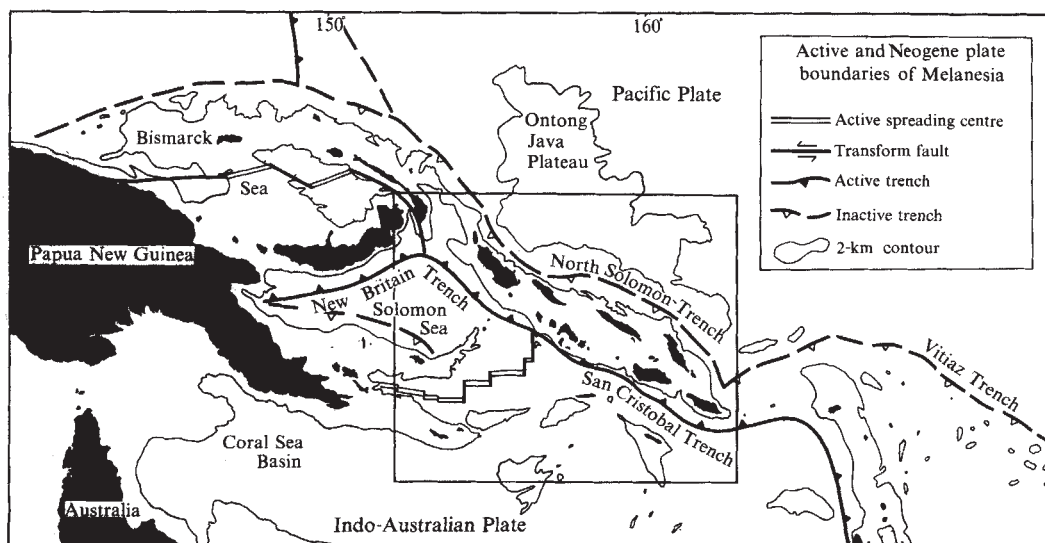


Fig. 1 Summary of active and Neogene plate boundaries of Melanesia (after ref. 19). Box shows location of Fig. 2.

Fig. 2 Regional seismicity ($ISC > 4.7 M_b$, 1 January 1964 to 30 June 1984) and Wadati-Benioff zone contours (at 50-km intervals) for the Solomon Islands. The Woodlark spreading system is shown following Taylor¹³ by double lines (spreading segments) and single lines (transforms). The dashed-dotted line marks the North Solomon trench; the dashed line indicates the Kaipito-Korighole fault. Large squares locate Plio-Pleistocene volcanoes; filled squares are active volcanoes.

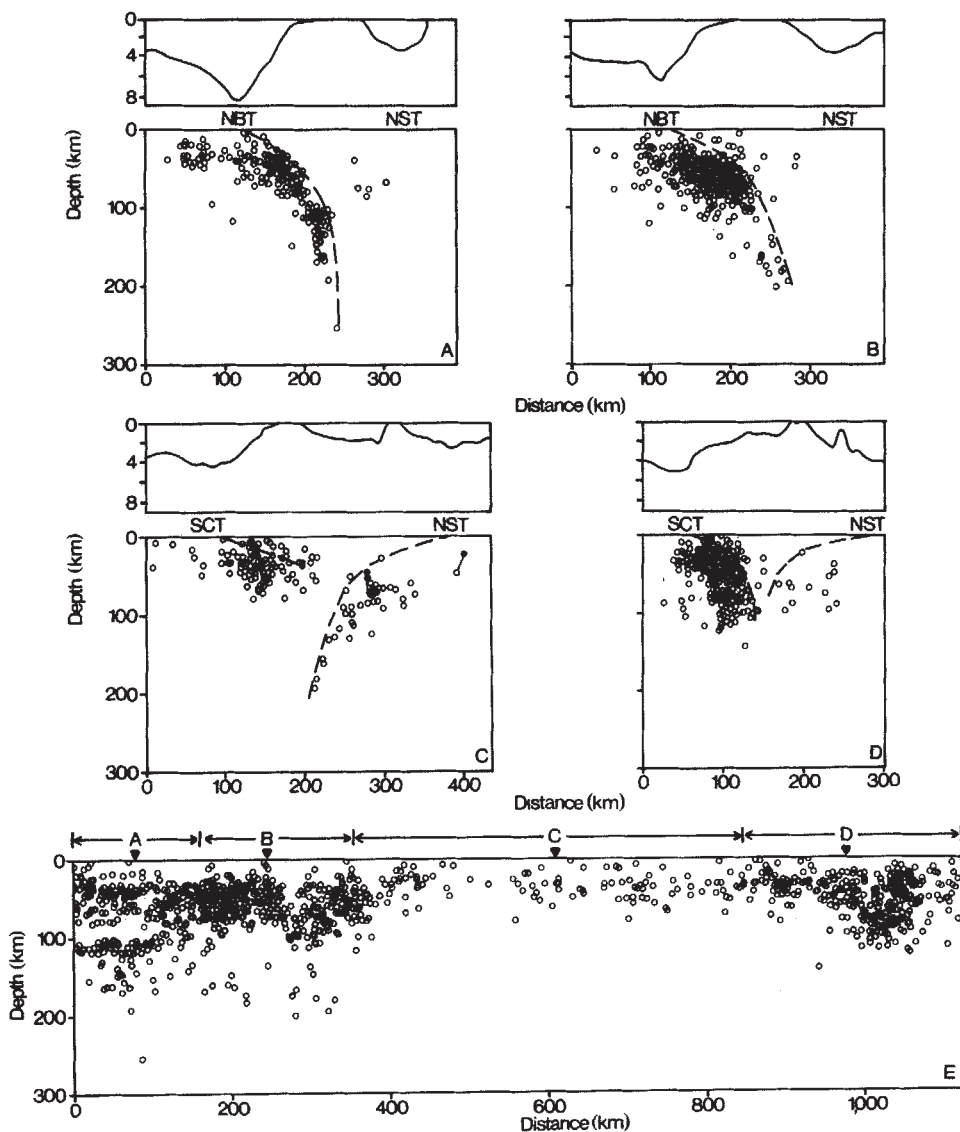
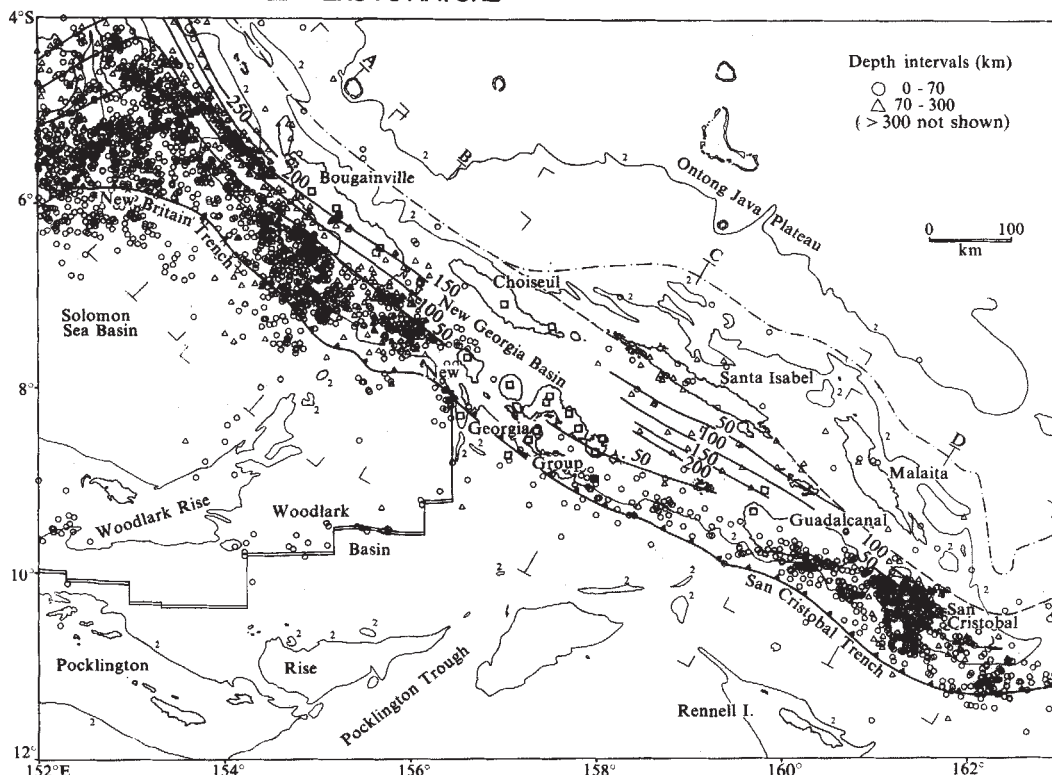


Fig. 3 Projections on vertical planes (located in Fig. 2) of ISC seismicity. The solid circle in C (top right, $\bullet$) represents a relocated earthquake. Dashed lines locate the inferred top of subducted lithosphere. Profile E is subparallel to the strike of the Solomon Islands, extends from 5.5°S, 153.5°E to 11.0°S, 162.0°E with a width of 425 km, and excludes the hypocentres associated with the south-west-dipping W-B zone. The New Britain, San Cristobal and North Solomon trenches are indicated by their initials.

plate. Near Bougainville (Fig. 3B) the dip of the seismic zone is less steep and the zone has become more diffuse. The W-B zone in these two profiles is continuous to just over 200 km, below which there is a seismic gap. The active volcanoes on Bougainville occur about 150 km above this W-B zone. Deep earthquakes occur throughout the Solomon Islands in cigar-shaped zones at depths of 400 ± 20 , 500 ± 20 and 535 ± 10 km, although there is little indication that the presently active W-B zone includes the deep seismicity.

Profile C (Fig. 3C) traverses the New Georgia Group. Subduction of the young lithosphere and active spreading system of the Woodlark Basin is characterized by shallow diffuse low-magnitude seismicity. Similar seismicity characteristics are observed in the region where the Chile Rise is being subducted beneath South America¹¹. There, the spreading segments trend parallel to the trench axis and, adjacent to the triple junction, there is a 3° gap in the chain of active volcanoes¹². In contrast, in the Solomon Islands the spreading segments trend sub-parallel to the convergence direction and, adjacent to the triple junction, there is a maximum in the volcanism of the island arc¹³. Although the seismicity east of the Woodlark Basin does not extend below 80 km, voluminous eruptions of Plio-Pleistocene lavas have formed the New Georgia Group and the active submarine volcano, Kavachi, within 30–90 km of the trench. Fig. 3D shows a north-east-dipping almost vertical seismic zone, continuous to just over 100 km, beneath San Cristobal. A relict south-west-dipping slab, discussed below, may force the presently subducting lithosphere into its almost vertical attitude.

A number of events in Fig. 3C, D define a south-west-dipping W-B zone that we attribute to the relict slab of Pacific Plate lithosphere formerly subducted at the NST. Several hypocentres in the shallow part of this zone were relocated by a joint hypocentral determination technique¹⁴ to depths significantly shallower than those reported by ISC and we note a similar systematic mislocation of events beneath the Japan Trench¹⁵. We have therefore drawn the top of the W-B zone above the apparent upper level of concentrated seismicity. Figure 3C clearly shows a south-west-dipping W-B zone. Curtis¹⁶, using data spanning 1961–70, noted that diffuse seismicity south of Santa Isabel seemed to form a south-west-dipping zone, but that it could conceivably be incorporated into a north-east-dipping W-B zone if the foci near New Georgia were included. Denham¹⁷ suggested that the Pacific Plate is underthrusting Santa Isabel; there are no cross-arc seismicity zones, however, to indicate that it is connected to the active plate boundaries to the south. Either the seismicity is entirely relict¹⁸, or the presently subducting lithosphere may be pushing the old slab deeper into the asthenosphere, forcing re-equilibration. It may be that the lack of intermediate earthquakes associated with the subduction of a spreading system beneath the central Solomon Islands affords us the unusual clarity in observing the relict slab.

The recognition that this relict slab is present has significant implications also for current petrological studies. Recent volcanism on Savo is located 100–150 km above the relict W-B zone and the Plio-Pleistocene volcanoes of Choiseul (Mt Matambe and Kambo Peak) are in a similar tectonic position along the strike of this W-B zone, from Savo (Fig. 2). These volcanoes may be evidence of a reactivation of magmatism associated with the relict slab.

Received 30 July; accepted 21 December 1984.

- McKenzie, D. P. *Geophys. J.* **18**, 1–32 (1969).
- Dewey, J. F. & Bird, J. M. *J. Geophys. Res.* **75**, 2625–2647 (1970).
- Johnson, T. & Molnar, P. *J. Geophys. Res.* **77**, 5000–5032 (1972).
- Coleman, P. J. & Packham, G. H. *Earth Sci. Rev.* **12**, 197–233 (1976).
- Weissel, J. K., Taylor, B. & Karner, G. D. *Tectonophysics* **87**, 253–277 (1982).
- Falvey, D. A. & Pritchard, T. in *Trans. Third Circum-Pacific Council Energy Mineral Resources* 593–599 (1984).
- Kroenke, L. W. *Cenozoic Tectonic Development of the Southwest Pacific (CCOP/SOPAC Technical Bull. 6, in the press)*.
- Brocher, T. in *Earth Science Ser. Circum-Pacific Council Energy Mineral Resources* (eds Brocher, T. & Holmes, R.) (in the press).
- Kroenke, L. W. thesis, Univ. Hawaii (1972).
- Coleman, P. J. *Pacif. Sci.* **24**, 289–314 (1970).
- Forsyth, D. W. *J. Geophys. Res.* **80**, 1429–1914 (1975).
- Herron, E. M., Cande, S. C. & Hall, B. R. in *Nazca Plate: Crustal Formation and Andean Convergence* (eds Kulm, L. D., Dymond, J., Dasch, E. J. & Hussong, D. M.) 683–701 (Geol. Soc. Am., Mem. 154, 1981).
- Taylor, B. in *Earth Science Ser. Circum-Pacific Council Energy Mineral Resources* (eds Taylor, B. & Exon, N.) (in the press).
- Dewey, J. W. thesis, Univ. California, Berkeley (1971).
- Hirata, N., Yamada, T., Shimamura, H., Inatani, H. & Suyehiro, K. *Geophys. J. R. astr. Soc.* **73**, 653–669 (1983).
- Curtis, J. W. *J. geol. Soc. Aust.* **20**, 1–19 (1973).
- Denham, D. *Bull. Aust. Explor. Geophys.* **6**, 78–79 (1975).
- Toksoz, N. *Sci. Am.* **233**, 88–98 (1975).
- Taylor, B. & Karner, G. D. *Rev. Geophys. Space Phys.* **21**, 1727–1741 (1983).

A 'northern oscillation' relating northern hemispheric pressure anomalies and the Indian summer monsoon?

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Analysis of the causes of the Indian monsoon have concentrated on climatological phenomena in the southern hemisphere, such as El Niño and the Southern Oscillation. By contrast, we have examined meteorological records for the northern hemisphere (0°–180°) spanning much of this century. We find that zonally-integrated mean sea-level pressure anomalies across Eurasia during January to April exhibit a statistically significant negative correlation between values for higher and sub-tropical latitudes. We further find that there is an association between above-normal activity of the Indian monsoon and a steep poleward-directed pressure anomaly gradient, which tends to persist through the summer and may indicate a strong zonal flow in the circumpolar westerlies. On the other hand, when this pressure anomaly gradient reverses, zonality is disturbed and the Indian monsoon tends to be drier than usual. Under these conditions, the circumpolar flow becomes persistently meridional as a result of a predisposition to blocking and splitting of the jet.

Exner¹ identified an inverse relationship between mean sea-level pressure in winter (December, January and February) over the northern hemispheric Eurasian Arctic and that in the central Mediterranean. Although he visualized a possible relationship with tropical precipitation, Exner did not pursue further analysis owing to the paucity of data. Through extensive correlation analysis, Walker^{2,3} related anomalies in the global centres of action, especially Icelandic and Aleutian, a few months (even a season) ahead with subsequent performance of the summer monsoon over India. Although Walker recognized the importance of Exner's analysis, he emphasized the pressure anomalies

Table 1 Correlation matrix of mean sea-level pressure anomaly zonally integrated across Eurasia (0–180°)

Lat. °N	80°	70°	60°	50°	40°	30°	25°
80°	1.00 (1.00)	0.77 (0.76)	0.32 (0.27)	–0.15 (–0.11)	–0.27* (–0.10)	–0.22* (0.01)	–0.20* (0.02)
70°		1.00 (1.00)	0.75 (0.71)	0.11 (0.06)	–0.32* (–0.26)†	–0.40* (–0.31)†	–0.38* (–0.30)†
60°			1.00 (1.00)	0.59 (0.59)	–0.09 (–0.03)	–0.39* (–0.40)*	–0.38* (–0.41)*
50°				1.00 (1.00)	0.57 (0.59)	0.05 (0.02)	–0.08 (–0.11)
40°					1.00 (1.00)	0.70 (0.64)	0.48 (0.45)
30°						1.00 (1.00)	0.91 (0.92)
25°							1.00 (1.00)

Upper line: January–April; 340 data points. Lower line (in parentheses): January + February + March + April; 85 data points.

* Significant at 0.1% level.

† Significant at 1% level.

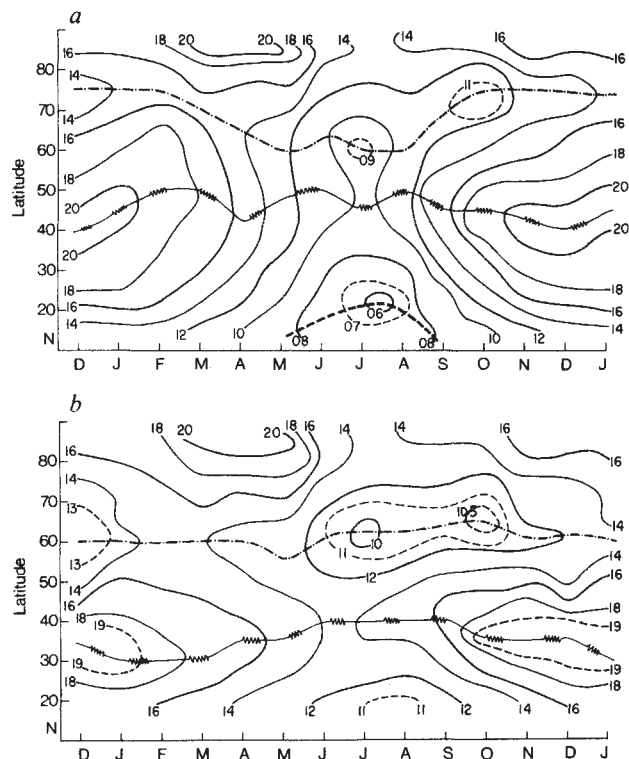


Fig. 1 Annual variation of the profile of mean sea-level pressure (mbar, first two digits omitted) over the northern hemisphere, zonally integrated: *a*, across Eurasia (0–180°; predominantly continental); *b*, around the northern hemisphere (oceanic and continental). Average for 85 yr (1900–84). —, Axis of sub-Arctic trough; ---, axis of sub-tropical ridge; ·····, axis of tropical trough.

in the centres of action of the southern hemisphere—the Southern Oscillation—and their teleconnections with Indian monsoon rainfall. Thereafter, particularly after Bjerknes⁴, more emphasis has been laid on the relationship between the Southern Oscillation/El Niño and the activity of the Indian summer monsoon. Ramage⁵ has drawn attention to the limitations of correlation analysis, in which a centre of action undergoes a significant change of location, resulting in a change of the sign of correlation. Therefore, we re-examine here the northern hemispheric pressure anomalies, especially those of continental Eurasia, with an analysis procedure that not only offsets distortions arising from positional changes in the centres of action but also filters sub-synoptic scale noise.

Mean sea-level pressure data for 26 yr (1951–76) received from the Chinese Academy of Sciences (Tao Shi Yan, personal communication), followed subsequently by more extensive data for 85 yr (1900–84) from the UK Meteorological Service formed the principal data sources for the study. Profiles of the monthly march of mean sea-level pressure, zonally integrated across Eurasia (0–180°; predominantly continental) and around the northern hemisphere (0–360°; oceanic and continental) in 5°-lat. steps from 85° N to 15° N are presented in Fig. 1. In the Eurasian profile (Fig. 1*a*), the sub-Arctic trough moves towards the Equator from 75° N in January to 60° N in July and has a higher value of mean pressure in winter compared with that in summer and autumn. The sub-tropical ridge located around 50° N in February/March shifts to 45° N in July and thence to 40° N in December. Again, the mean pressure values are higher in winter than in summer. In the average profile, the sub-tropical high is substantially effaced and is seen as a weak ridge in summer at sea level. The axis of the sub-Arctic trough approaches the position of the weak sub-tropical high belt during May–August. The tropical trough is sharply delineated, traversing from 15° N in May to near 25° N in July/August and shifting equatorward

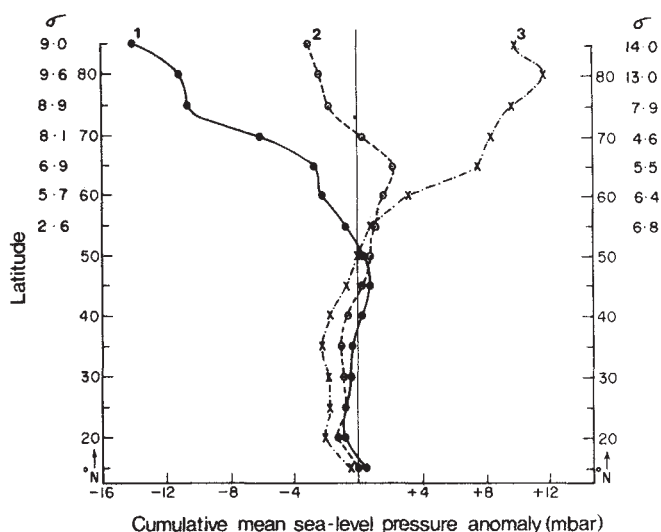


Fig. 2 Latitudinal profile of cumulative anomaly of mean sea-level pressure (January–April), zonally averaged across Eurasia (0–180°) and composited for three categories of performance of the Indian summer monsoon: (1) above normal ($\geq +6\%$), 12 yr; (2) normal ($\geq -5\%$ to $\leq +5\%$), 18 yr; (3) below normal ($\leq -6\%$), 12 yr. Standard deviation (σ) of the two contrasting samples is given with respect to the latitude, on either side. The two samples are significantly different. Note the following: (i) rainfall departures are available for north-west India and the Peninsula for the period 1940–81. (ii) In the almost 100-yr data set supplied by the UK Meteorological Service, data gaps have been reported over USSR between 1916 and 1921 and 1938–39. Hence, the data-gap-free period of 42 yr (1940–81) was chosen for preparation of the composites. (iii) 5% is half of the standard deviation of long-term rainfall of India.

thereafter. In contrast, the axes of the sub-Arctic trough and the sub-tropical ridge in the hemispherical profile (Fig. 1*b*) are about 5–15 degrees of latitude equatorward. The tropical trough is relatively less well defined.

Space-time sections of Eurasian and hemispherical averages of mean sea-level pressure anomalies (January 1900–April 1984) gave the first indication of periodic reversals of pressure anomalies, particularly in winter and early spring, over the Arctic, poleward of 55° N. To express this quantitatively, a correlation matrix (Table 1) compiled with pressure anomaly values from January to April, serially and cumulatively is presented. A marked inverse relationship is noticed between pressure anomalies over higher/middle latitudes and those of the sub-tropics/tropics. The relationship is statistically significant at the 0.1% level. The negative association is striking between 70°/60° N and 30°/25° N. The matrix further suggests that a similar inverse relationship exists between pressure anomalies of the tropics of the northern and the southern hemispheres respectively.

To look for an association with the performance of the summer monsoon over India, the 42-yr data-gap-free period 1940–81 was chosen from the almost 100-yr sample. Monsoon seasonal rainfall departures (June–September) from the long-term norm for the plains of India, north-west India and the Peninsula are used to group the 42-yr period into three categories of monsoon performance over India, namely, 12 yr each of above normal ($\geq +6\%$) and below normal ($\leq -6\%$) and 18 normal years ($\geq -5\%$ to $\leq +5\%$). (Note that 5% represents half the standard deviation of long-term monsoon rainfall.)

The composited cumulative pressure anomaly profiles (January–April) display contrasting characteristics (Fig. 2). During this season, preceding above-normal monsoon performance, a distinct negative anomaly exists, with a steep gradient of ~ 15 mbar directed poleward from the domain of the sub-tropical

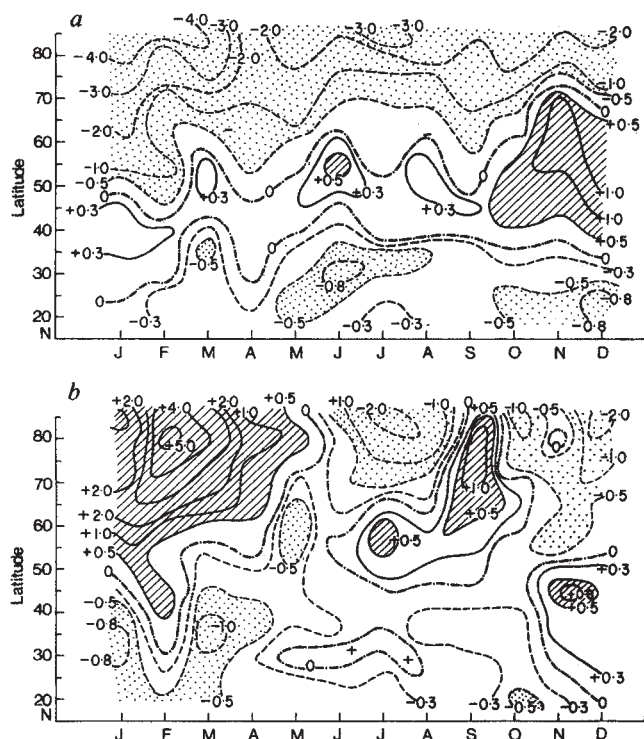


Fig. 3 Monthly march of mean sea-level pressure anomaly, zonally averaged across Eurasia ($0-180^\circ$) and composited for: *a*, above normal monsoon ($\geq +6\%$, 12 yr); *b*, below normal monsoon ($\leq -6\%$, 12 yr). Note the following: (i) in *a* isopleths of negative and positive anomaly run zonally; in *b* isopleths of pressure anomaly display a meridional orientation. (ii) Positive rainfall anomaly during above-normal (good) monsoon years is $\sim +6-+8\%$, the largest being $+18\%$ in 1961. However, during below-normal (aberrant) monsoon years, rainfall departure is appreciably negative, ranging from -12% to -33% . (iii) Positive mean sea-level pressure anomaly over the Arctic poleward of 45°N is conspicuously large during January–April in highly aberrant monsoon years with rainfall anomaly $< -16\%$ of normal. (iv) Note the contrasting pressure anomaly distribution in summer, equatorward of 40°N , in relation to the opposing pressure anomaly over middle and higher latitudes during January–April.

ridge (45°N). On the other hand, when the summer monsoon performance is below normal, the antecedent anomaly pattern reverses and is diametrically opposite; the gradient of pressure anomaly of $\sim 13\text{ mbar}$, is now directed equatorward from the Arctic to 45°N . The curve antecedent to normal monsoon behaviour lies in-between the two contrasting profiles. The standard deviation (σ) of the two 12-yr samples, noted on either side of Fig. 2, shows a general decreasing tendency from the Arctic (85°N) to the middle latitude (55°N); the associated standard error is small. The mean $\pm\sigma/2$ values show that the two samples retain their significantly dissimilar characteristics. Application of 'Students' *t* test confirms that the two samples are statistically significant, poleward of latitude 60°N , with $P < 0.05$.

Using the entire series (1900–84), profiles were constructed with pressure anomalies averaged across Eurasia and around the northern hemisphere corresponding to 19 above-normal and 19 below-normal monsoon years. These two sets of profiles display characteristics essentially similar to those in Fig. 2 as well as statistical tests that are significant.

Figure 3 presents a composite of monthly march of pressure anomaly across Eurasia for extremes of monsoon performance. In the above-normal monsoon composite (Fig. 3*a*), a conspicuously large negative pressure anomaly is observed over the Arctic region poleward of 40°N in the preceding winter and early spring (January–April), persisting through the summer months and even thereafter. Immediately equatorward, the sub-

tropical high is enhanced, but in the tropical trough region, equatorward of 40°N , during the ensuing summer (especially June–August) a negative anomaly, almost reaching $\sim -1.0\text{ mbar}$ is observed. Considering that the composite is an average of 12 above-normal years and covers one half of the northern hemisphere, a negative pressure anomaly of this magnitude at low latitude is significant. The associated summer monsoon turns out to be active.

However, in the below-normal monsoon composite (Fig. 3*b*), the pronounced positive pressure anomaly of up to $+5\text{ mbar}$ during January–April suggests a weakened Arctic trough. The sub-tropical ridge is totally annihilated and, in the ensuing summer, the tropical trough, equatorward of 40°N , is now characterized by a weak positive pressure anomaly. The associated summer monsoon activity is naturally suppressed.

Composites for contrasting monsoon years, prepared with anomalies of 500-mbar topography integrated across Eurasia ($0-180^\circ$) and around the northern hemisphere, using data for the period 1951–79^{6,7}, also display characteristics similar to those in Fig. 3. A significantly large positive topographic anomaly overlies a positive mean sea-level pressure anomaly and vice versa.

Blocking, over high latitudes of Eurasia, was recently associated with monsoon droughts over India^{8,9}. Arising from dynamical causes, the relatively more-frequent antecedent blocking, over west Eurasian high latitudes, was observed to extend from the lower stratosphere to sea level. Painting¹⁰ reported increased blocking during winter in association with a positive mean sea-level pressure anomaly over high latitudes of the northern hemisphere. The positive pressure anomaly during January–April (Fig. 3*b*) could, therefore, be attributed to persistent high-latitude blocking over Eurasia. Marked differences were reported in hemispherical circulation characteristics over higher latitudes and the tropics during August, the typical summer month, in years of contrasting monsoon performance⁸. Thus, contrasting winter and early spring abnormality in pressure at mean sea level and in the overlying troposphere of the middle and higher latitudes of Eurasia seems to predetermine, to a great extent, the probable pressure anomaly distribution over low-latitude tropics of south Asia in the ensuing summer and the associated monsoon performance. A simple linear relationship does not, however, emerge between the magnitudes of the negative/positive pressure anomalies before the monsoon and subsequent monsoon rainfall departure. Work in progress to compile a suitable pressure oscillation index for the Eurasian region with the long-time pressure data series may provide further insights into this complex problem.

This study does not presume to preclude the apparent effects of El Niño or the Southern Oscillation on the behaviour of the summer monsoon over the Indian subcontinent. The role of soil moisture over high ground of south Asia and the contribution of sensible heat could be among the other factors leading to the organization of the monsoon trough (D. E. Jones and C. K. Folland, personal communication). This investigation just suggests that interaction may exist between antecedent pressure anomalies over middle and high latitudes of the northern hemisphere—the Northern Oscillation?—and the Indian summer monsoon. Much further work remains to be done.

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1. Exner, F. M. *Sitz.d.k.Akad. der Wissenschaften, Wien CXXII*, 1165–1240 (1913).
2. Walker, G. T. *Mem. India Met. Dep.* 24(IV), 75–131 (1923).
3. Walker, G. T. *Mem. India Met. Dep.* 24(IX and X), 275–345 (1924).
4. Bjerknes, J. *Mon. Weath. Rev.* 97, 167–172 (1969).
5. Ramage, C. S. *J. Clim.* 3, 223–231 (1983).
6. *Die Grosswetterlagen Europas* (Deutscher Wetterdienst, Offenbach, 1953–79).
7. *Nordhemispherische Wetterlage* (Beilage zur Berliner Wetterkarte, 1960–79).
8. Raman, C. R. V. & Rao, Y. P. *Nature* 289, 271–273 (1981).
9. Raman, C. R. V. *Proc. Monex Symp. Bali* (WMO) 1, 21–25 (1982).
10. Painting, D. J. *Met. Off., Lond., Sci. Pap. No. 35*, 1–25 (1977).

Stable isotope data and depositional environments in the late Quaternary Arctic Ocean

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There has been much speculation about the history of the Arctic Ocean, particularly its response to the late Quaternary climatic fluctuations¹⁻⁸. As a result, considerable data have been gathered from Arctic Ocean sediment cores to reconstruct glacial and interglacial Arctic Ocean palaeoenvironments^{8,9}. But even with these data the reconstructions and the correlations with the Quaternary chronostratigraphy have been unsatisfactory, mainly because of the lack of detailed stratigraphical data such as those provided by stable oxygen-isotope stratigraphy¹⁰. Here we present stable isotope records from Arctic Ocean sediment cores which can be correlated convincingly with corresponding data from the North Atlantic. Together with lithostratigraphical data, they provide new evidence on Arctic Ocean history in relation to global late Quaternary climatic fluctuations.

Our two sediment cores were collected during the Fram-I expedition¹¹. Core Fram-I/4 was raised from the Fram Basin (84°29'56" N, 08°58'39" W, water depth 3,820 m) and core Fram-I/7 from the northern flank of Nansen Ridge (83°52'38" N, 06°57'17" W, water depth 2,990 m) (Fig. 1). The stable isotope measurements were made on planktonic foraminiferal shells of sinistrally coiled *Neogloboquadrina pachyderma*, with samples containing 55 individuals on average. Measurements followed standard procedures described elsewhere^{12,13}. The O-isotope records in the upper parts of both cores (Fig. 2) offer clear evidence of the last glacial-interglacial transition, called Termination I (ref. 14), which is subdivided into Terminations I_A and I_B as defined by Duplessy *et al.*¹⁵. Additionally, the marked increase in $\delta^{18}\text{O}$ in core Fram-I/4 just below Termination I_B may correspond to the Younger Dryas¹⁶. The difference between the $\delta^{18}\text{O}$ shifts at the level of Terminations I_A and I_B in the two cores (Table 1) may be a result of different sampling intervals, that is, the larger sampling intervals of core Fram-I/7 may have resulted in a smoothing of the $\delta^{18}\text{O}$ record. On the other hand, bioturbation effects cannot be excluded. Nevertheless, the absolute shifts in $\delta^{18}\text{O}$ of $\sim 2.7\%$ for the whole of Termination I in both cores exceed the actual 'ice-effected' value of 1.6–1.8%¹⁷. This $\sim 1\%$ excess may indicate a temperature increase of 4°C. More probably, however, we may assume there was a direct supply of meltwaters derived from the Arctic ice during

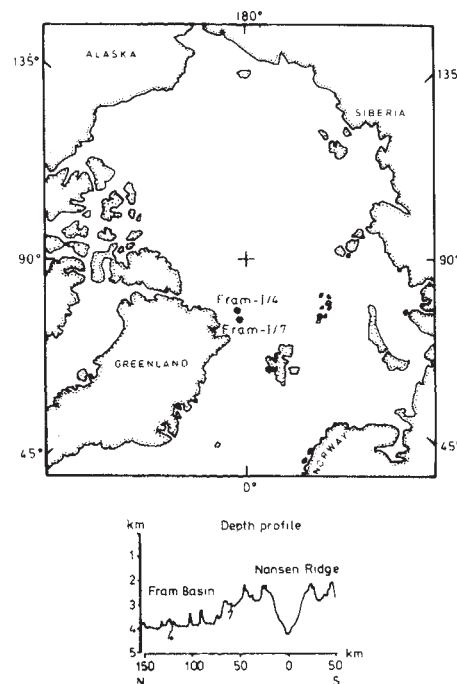


Fig. 1 Locations of cores Fram-I/4 and -I/7. The depth profile traces the bathymetry under the drift of ice station 'FRAM-I'.

deglacial warming, which would indicate a decrease of salinity of $\sim 0.6\%$ ¹⁸.

Furthermore, both cores exhibit a pronounced trend towards lighter $\delta^{18}\text{O}$ values in their lower parts, which may represent the O-isotope stage boundary 2/3. The strongly negative $\delta^{18}\text{O}$ peak during stage 2 in core Fram-I/7, however, is hard to explain in terms of global warming and its absence from the $\delta^{18}\text{O}$ record of core Fram-I/4 may indicate the presence of displaced sediments. Such sediments may have been brought in by turbidity currents, which are likely to occur on flanks of submarine ridges like the one from which core Fram-I/7 was raised (Fig. 1).

At present there are no ^{14}C data for either Fram core, but the O-isotope records resemble those of cores from low-latitude locations^{10,19}. Therefore, because of this and the relatively short oceanic mixing time, it seems feasible to transfer the chronostratigraphical fixpoints from ^{14}C -dated low-latitude cores^{15,20-22} to the O-isotope analogues of cores Fram-I/4 and Fram-I/7 (Fig. 2, Table 1). The average sedimentation rates between the top of Termination I_B and the stage boundary 2/3, which can be calculated from these fixpoints, are ~ 1.8 and $\sim 2.6 \text{ cm} \times 10^{-3} \text{ yr}$ for cores Fram-I/4 and Fram-I/7, respectively, and

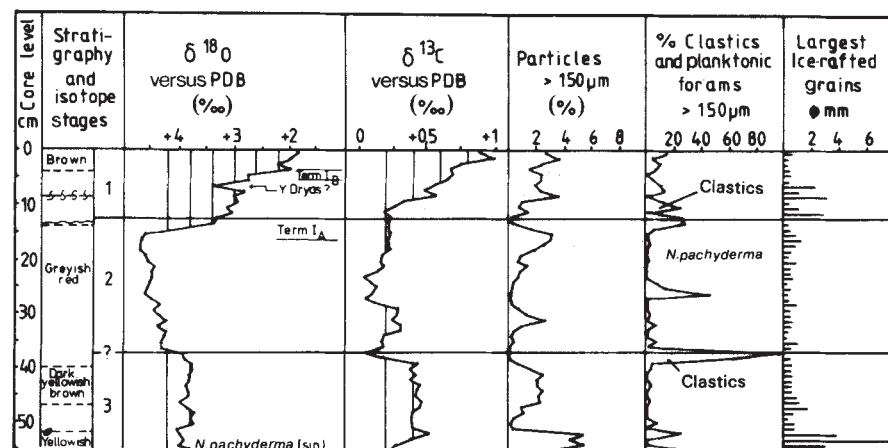
Table 1 Chronostratigraphy, $\delta^{18}\text{O}$ shifts and sedimentation rates of cores Fram-I/4 and -I/7

Termination I	Fram-I/4				Fram-I/7		
	¹⁴ C age (kyr)	Core depth (cm)	$\delta^{18}\text{O}$ shift (%)	Sedimentation rates (cm kyr ⁻¹)	Core depth (cm)	$\delta^{18}\text{O}$ shift (%)	Sedimentation rates (cm kyr ⁻¹)
Termination I _B	8.5	3.0			13.0		
	10.0	5.0(?)	+0.75	1.33	17.0	+1.92	2.67
Younger Dryas			-0.56	2.25			
	10.8	6.8(?)					
Termination I _A	13.5	12.0	+1.33	1.60	23.0	+0.59	1.20
	16.0	16.0			26.0		
O-Isotope stage boundary							
1/2	13.5 ²⁰	12.0	+2.67	1.74	23.0	+2.75	1.73
			(for entire Term. I)			(for entire Term. I)	
2/3	27.0 ²¹	36.5(?)	-0.41	1.89	79.5(?)	-0.57	3.37*
			(for stage 2)			(for stage 2)	

Data for Termination I are from refs 17, 22.

* Stage 2 sedimentation rates in core Fram-I/7 were estimated without a section between 54 and 65 cm, which is suggested to represent displaced sediments (see text).

FRAM-I/4



FRAM-I/7

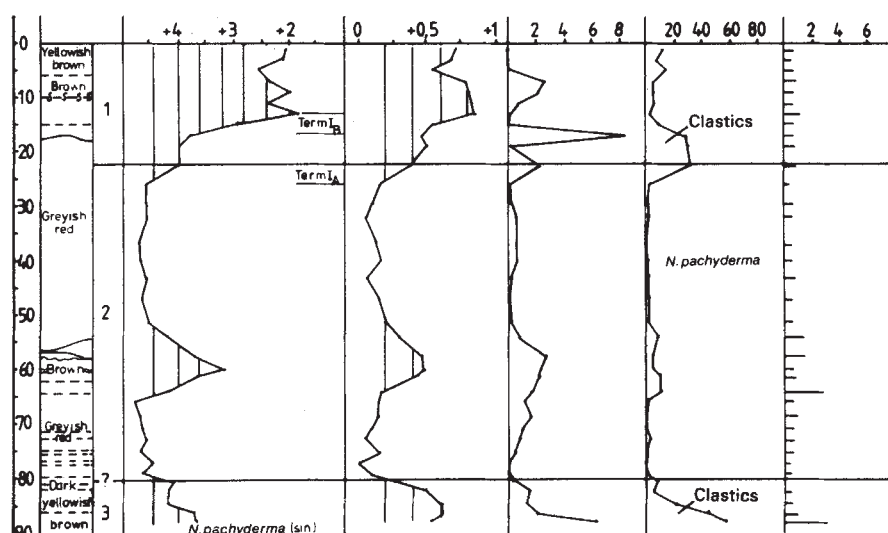


Fig. 2 Stable isotope records and lithostratigraphies of cores Fram-I/4 and -I/7. $\delta^{18}\text{O}$ values are shown relative to the PDB reference standard.

much higher than those previously reported for central Arctic Ocean sediments^{23,24}. However, sedimentation rates of a similar order of magnitude have been reported recently²⁵.

Finally, the similarity in O-isotope data between our Arctic Ocean cores and those from the adjacent Norwegian-Greenland Sea^{26,27} should be mentioned (Fig. 3). During stage 2 the O-isotope curves actually coincide without any significant difference, suggesting similar surface-water conditions in both deep-sea basins. During O-isotope stages 3 and 1, the Arctic cores tend towards lighter $\delta^{18}\text{O}$ values, perhaps because of a more pronounced meltwater effect in the Arctic Ocean than in the Norwegian-Greenland Sea.

The most prominent feature in the $\delta^{13}\text{C}$ records of both cores is the steady increase towards heavier values during Termination I (Fig. 2). This increase seems to start somewhat earlier in core Fram-I/7 than in core Fram-I/4, probably because of the different sampling intervals applied on the cores; stage 3 also exhibits heavier values than stage 2. The distinct increase in $\delta^{13}\text{C}$ in core Fram-I/7 during stage 2, as well as the $\delta^{18}\text{O}$ decrease in the same core section described above, does not occur in the $\delta^{13}\text{C}$ record of core Fram-I/4; this may support the assumption that this part of the section has been affected by sediment displacement.

Because $\delta^{13}\text{C}$ variations generally follow variations in hydrological parameters such as O_2 content²⁸, the increasing ^{13}C content in planktonic *N. pachyderma* (sin) shells may indicate the penetration of comparatively well-oxygenated surface-subsurface waters into this general region. This suggests that present-day circulation patterns in the Arctic Ocean may have

begun during Termination I, a suggestion strongly supported by $\delta^{13}\text{C}$ values of *N. pachyderma* (sin) from Norwegian Sea cores²⁹ where a similar increase starts somewhat before Termination I. This may reflect the retreat of the glacial-stage ice cover with succeeding inflow of temperate North Atlantic Drift water into the Norwegian-Greenland Sea and, with respect to the $\delta^{13}\text{C}$ signal in our cores, into the Arctic Ocean through the Fram Strait³⁰. Even the strong $\delta^{18}\text{O}$ shift in both cores during Termination I may in part be attributable to an increase in water temperature, indicating the influence of these warm waters.

The sediment compositions of the two cores correspond closely with their stratigraphy, permitting a correlation of their lithostratigraphies also. The lithologies may briefly be characterized as follows: the Upper Holocene sediments contain small amounts of CaCO_3 and coarse-grained ice-rafted material, whereas in the sediments, which were deposited during Termination I, that is, the global deglaciation, concentrations of these components are markedly increased. In the deposits corresponding to O-isotope stage 2, that is, fully glacial conditions, coarse ice-rafted material is less abundant, whereas concentrations of CaCO_3 are equal to or even higher than those in the Holocene sediments. Only in the section of core Fram-I/7, which has already been suggested to represent displaced sediments, are amounts of both components increasing. In the assumed O-isotope stage 3 sediments, the proportions of the coarse fractions and the amounts and grain sizes of ice-rafted material are clearly increasing, whereas concentrations of CaCO_3 are decreasing. The coarse fractions throughout both sediment cores consist mainly of planktonic foraminifers (mainly *N. pachyderma* (sin))

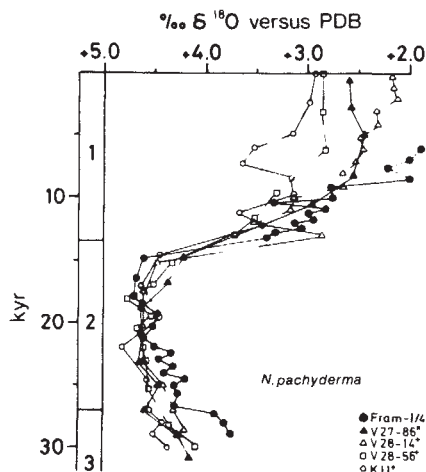


Fig. 3 O-isotope curves of cores from Norwegian-Greenland Sea^{26,27} and of core Fram-1/4. A better comparison has been obtained by replotting the O-isotope data on a timescale defined by well-established radiocarbon ages^{15,20-22}.

throughout all units, with smaller amounts of benthic fossils such as foraminifers, sponges and ostracods. The sedimentological and micropalaeontological data will be presented in more detail elsewhere³¹.

The most important results and implications from the present study may be summarized as follows. (1) Sedimentation rates in the Arctic Ocean may be an order of magnitude higher than was previously assumed. (2) Increased amounts of ice-rafted material accumulated during transitions towards both colder and warmer climates, but during fully glacial and interglacial climates the supply of this component was strongly diminished. (3) The presence of planktonic foraminifers throughout both cores records a modest productivity of surface-subsurface waters even during fully glacial conditions. (4) The reddish-brown colours of the sediments, as well as the benthic fossils found throughout both cores, may be evidence of an efficient renewal of bottom water masses in the eastern Arctic Basin throughout the late Quaternary. In contrast, however, the $\delta^{13}\text{C}$ records suggest a distinct change in oceanic circulation during Termination I towards present-day circulation patterns. (5) The obvious correspondence of the O-isotope records presented here with those from low-latitude sediment cores may indicate an active and efficient exchange of Arctic Ocean water masses with those of the global ocean throughout the late Quaternary. Furthermore, the close agreement in O-isotope data from our Arctic Ocean cores and those from the Norwegian-Greenland Sea^{26,27} during stage 2 suggests similar hydrographies of surface-subsurface waters in both deep-sea basins during fully glacial conditions.

Fram-I cores were collected under the supervision of Y. Kristoffersen. Isotope measurements were made at the ^{14}C laboratory of the University of Kiel. We thank M. Sarnthein, H. Erlenkeuser, G. Miller and N. Shackleton for discussions. This study was supported by the US Office of Naval Research, the Norwegian Polar Institute and the German Research Program on Climate.

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- Donn, W. L. & Ewing, M. *Science* **152**, 1706-1712 (1966).
- Donn, W. L. & Shaw, D. M. *Progr. Oceanogr.* **4**, 105-113 (1967).
- Olausson, E. *Geol. Förel. Stockh. Förh.* **91**, 185-200 (1969).
- Ewing, M. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 565-573 (Yale University Press, 1971).
- Olausson, E. *Ambio spec. Rep.* **2**, 13-17 (1972).
- Hughes, T., Denton, G. D. & Grosswald, M. G. *Nature* **266**, 596-602 (1977).
- Herman, Y. & Hopkins, D. M. *Science* **209**, 557-562 (1980).
- Clark, D. L. *Nature* **300**, 321-325 (1982).
- Clark, D. L., Whitman, R. R., Morgan, K. A. & Mackey, S. D. *Geol. Soc. Am. spec. Pap.* **181** (1980).
- Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39-55 (1973).
- Kristoffersen, Y. *Isdriftstasjonens Fram-I Ekspedisjonsrapport* (Norwegian Polar Institute, Oslo, 1979).

- Lutze, G.-F. *et al. Init. Rep. DSDP* **47**, 727-739 (1979).
- Ganssen, G. & Sarnthein, M. in *Coastal Upwelling: Its Sediment Record* (eds Suess, E. & Thiede, J.) 99-121 (Plenum, New York, 1983).
- Broecker, W. S. & Van Donk, J. *Rev. Geophys. Space Phys.* **8**, 169 (1970).
- Duplessy, J. C., Delibrias, G., Turon, J. L., Pujol, C. & Duprat, J. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **35**, 121-144 (1981).
- Berglund, B. E. *Boreas* **8**, 98-118 (1979).
- Duplessy, J. C. in *Climatic Change* (ed. Gribbin, J.) 46-67 (Cambridge University Press, 1978).
- Craig, H. & Gordon, L. I. in *Stable Isotopes in Oceanographic Studies and Paleotemperatures* (ed. Tongiorgi, E.) 9-130 (Consiglio Nazionale delle Ricerche, Laboratorio di Geologia Nucleare, Pisa, 1965).
- Emiliani, C. & Shackleton, N. J. *Science* **183**, 511-514 (1974).
- Koopmann, B. *Forsch. Ergebn. C35*, 23-59 (1981).
- Morley, J. J. & Hays, J. D. *Earth planet. Sci. Lett.* **53**, 279-295 (1981).
- Sarnthein, M., Erlenkeuser, H. & Zahn, R. *Bull. Inst. Geol. Bassin d' Aquitaine* **31**, 393-407 (1982).
- Ku, T. L. & Broecker, W. S. *Progr. Oceanogr.* **4**, 95-105 (1967).
- Clark, D. L., Vincent, J.-S., Jones, G. A. & Morris, W. A. *Nature* **311**, 147-149 (1984).
- Sejrup, H. P., Miller, G. H., Brigham-Grette, J., Lövlie, R. & Hopkins, D. *Nature* **310**, 772-775 (1984).
- Kellogg, T. B., Duplessy, J. C. & Shackleton, N. J. *Boreas* **7**, 61-73 (1978).
- Streeter, S. S., Belanger, P. E., Kellogg, T. B. & Duplessy, J. C. *Quat. Res.* **18**, 72-90 (1982).
- Kroopnick, P. *Earth planet. Sci. Lett.* **49**, 469-484 (1980).
- Sejrup, H. P., Jansen, E., Erlenkeuser, H. & Holte Dahl, H. *Quat. Res.* **21**, 74-84 (1984).
- Gammelsrod, T. & Rudels, B. *Polar Res.* **1**, 115-126 (1983).
- Markussen, B., Zahn, R. & Thiede, J. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* (in the press).

Microhabitats of benthic foraminifera within deep-sea sediments

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Benthic foraminifera are protozoans found throughout the deep-sea environment, secreting a test of calcium carbonate or constructing a test of cemented sediment particles (agglutinated or arenaceous foraminifera). In typical deep-sea sediments, the organic cement of agglutinated taxa degrades upon burial in the sediment and, consequently, few specimens survive in the fossil record. In contrast, calcareous species are well preserved in most oceanic sediments, except at abyssal depths where most carbonate sediment is dissolved because of high levels of carbonate undersaturation of the bottom waters. Although benthic foraminifera have been widely used in studies of Cenozoic palaeoceanography, little is known about the ecology of deep-sea species. I present here an analysis of living (stained) benthic foraminifera within the upper 15 cm of deep-sea sediments, which reveals species-specific microhabitat preferences, with distinct morphological features found with epifaunal and infaunal species. The existence of infaunal habitats suggests that the distribution of certain foraminifera is not directly controlled by overlying bottom-water conditions, but by physicochemical conditions within the sediments. The microhabitat preferences may also explain interspecific carbon isotope differences, as existing data show that infaunal foraminifera generally have lower $\delta^{13}\text{C}$ isotope values than epifaunal species.

Early distributional studies demonstrated that deep-sea benthic foraminiferal assemblages are depth related¹ and this information was used in palaeoenvironmental studies of marine sedimentary sequences to estimate past water depths^{2,3}. More recent studies have shown that deep-sea benthic foraminiferal faunal assemblages are related to the distribution of distinct water masses in the deep ocean, although the variables which control the faunal patterns are largely unknown⁴. In addition to faunal distribution studies, oxygen and carbon isotope analyses of benthic foraminifera have been used extensively to estimate polar ice volumes^{5,6} and to study regional palaeocirculation conditions in the deep ocean⁷⁻⁹.

A number of unanswered questions remain which limit the usefulness of the faunal and isotope data. Although significant correlations between bottom-water hydrographic variables (for

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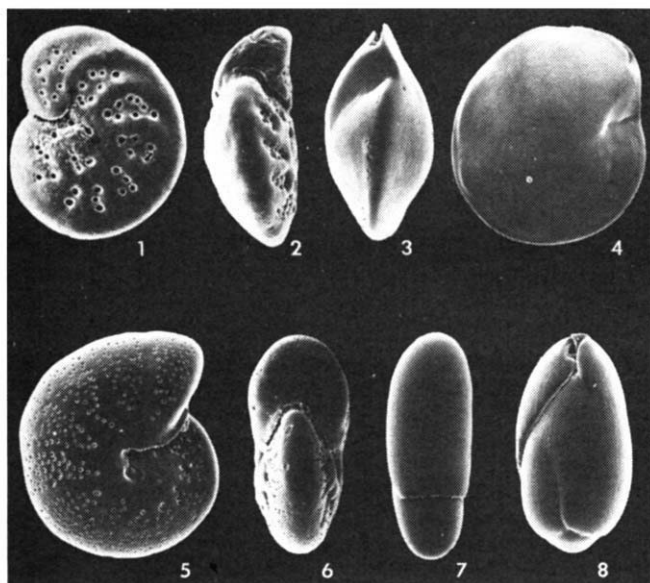


Fig. 1 Illustrations of important living deep-sea benthic foraminifera in OC-86/2-7-4. (1) *Planulina wuellerstorfi*, umbilical view ($\times 103$); (2) *P. wuellerstorfi*, edge view ($\times 79$). Pores can be seen on the umbilical side but are absent from the spiral side. (3) *Hoeglundina elegans*, edge view ($\times 55$); (4) *H. elegans*, umbilical view ($\times 44$); (5) *Melonis barleeanum*, side view ($\times 82$); (6) *M. barleeanum*, edge view ($\times 72$); (7) *Chilostomella oolina* ($\times 51$); (8) *Globobulimina affinis* ($\times 51$). Small pores are present over most of the test surface of *C. oolina* and *G. affinis*. The epifaunal species (1-4) have plano-convex or biconvex shapes with surface pores on only one side or absent, whereas the infaunal species (5-8) have more rounded tests with surface pores evenly distributed over most of the test surface.

example, temperature, salinity, dissolved oxygen) and faunal data do exist in regional studies, these correlations are not consistent over broad areas of the ocean, indicating that other variables are controlling faunal patterns. Analysis of the carbon isotope composition of benthic foraminiferal species shows that there are differences (frequently referred to as 'vital effects') between species, with some species secreting calcium carbonate in carbon isotope disequilibrium with the overlying bottom water¹⁰⁻¹³. Interspecific differences in $\delta^{13}\text{C}$ have led to speculation that certain species of deep-sea benthic foraminifera have infaunal microhabitats within the sediment where pore-water $\delta^{13}\text{C}$ influences carbonate $\delta^{13}\text{C}$ (refs 10-12). Palaeoceanographic studies often use time-series data from more than one species with disequilibrium factors applied to correct for species differences, with the assumption that these interspecific differences are constant through time. However, a number of studies have shown that for some species these fractionation factors are variable in the Cenozoic record^{14,15}.

To gain insight into these questions, two box cores were taken from the western North Atlantic on R/V *Oceanus* cruise 86, leg 2 (OC-86/2), in September 1980, using an Ocean Instruments Mark III box corer (0.25-m² surface area) divided into 25 10-cm $\times$ 10-cm subsamples. OC-86/2 box core 7-4 (OC-86/2-7-4) was taken at 38°12.9'N, 71°30.4'W in 3,000-m water depth on the eastern US continental rise and *Oceanus* 86/2 box core 5-3 was taken at 35°22.8'N, 65°20'W in 4,800-m water depth on the Bermuda Rise. Both box cores had clear water above the surface indicating the surface sediments were undisturbed during the coring process. Slices (1 cm thick) of two subsamples from OC-86/2-7-4 were taken from the sediment/water interface to 15 cm depth, and from one subsample in OC-86/2-5-3 to 9 cm depth. Each 100-cm³ sample was preserved in 150 ml of buffered 4% formalin solution and the samples were treated with Rose Bengal stain to identify benthic foraminifera living at the time

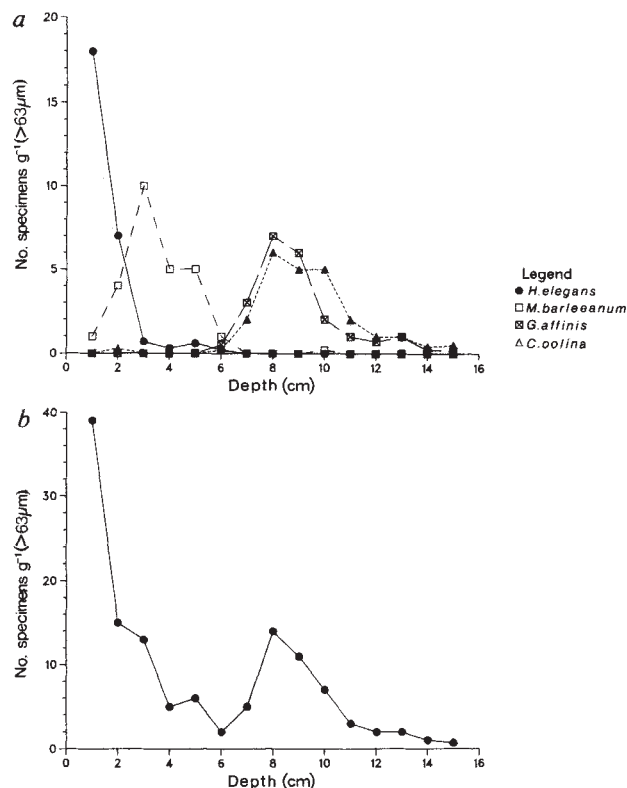


Fig. 2 Summary of benthic foraminiferal data from OC-86/2-7-4. *a*, Number of specimens ($>150\ \mu\text{m}$) per g of $>63\text{-}\mu\text{m}$ sediment of four dominant species; *b*, total number of specimens ($>150\ \mu\text{m}$) per g of $>63\text{-}\mu\text{m}$ sediment. The species are vertically stratified within the deep-sea sediments with a majority of the living fauna (73%) found below 2 cm.

of collection. In the laboratory, each sample was washed over a 63- μm sieve, the $>63\text{-}\mu\text{m}$ fraction was dried, weighed and dry sieved over a 150- μm sieve. All of the living (stained) benthic foraminifera were picked from the $>150\text{-}\mu\text{m}$ sample, sorted by species and counted. Rose Bengal is a biological stain that absorbs on the surface of protoplasm and has been widely used in studies of living benthic foraminifera. This method does have limitations (see ref. 16). A living individual is recognized by the presence of a bright red or violet coloration, within at least one chamber of the test, that can be seen in species with a hyaline test wall. Agglutinated foraminifera with test walls composed of cemented particles or miliolids that have an opaque porcellaneous calcareous wall were not considered here because it was difficult to observe stained protoplasm within the interior portion of the test. Some specimens had a tint of pink colour owing to the presence of an organic membrane, or spots of red colour reflecting particles of organic matter, but these were not counted as living specimens.

The dominant living species in one subsample of core 7-4 are illustrated in Fig. 1 and plotted in Fig. 2*a*, where the data are expressed as the number of living specimens ($>150\ \mu\text{m}$) per gram of $>63\text{-}\mu\text{m}$ dry sediment (Table 1). (Results from a second subsample are almost identical and are not presented here.) In the top 2 cm of sediment, *Hoeglundina elegans* (d'Orbigny) is the dominant species and is rare at 2-5 cm. Additional species within the top 1 cm include *Cibicides kullenbergi* (Parker), *Oridorsalis tener* (Brady) and *Planulina wuellerstorfi* (Schwager). Within the 1-6-cm interval, *Melonis barleeanum* (Williamson) is the dominant species with a maximum of 8 specimens per g in the 2-3-cm interval. From 6-7 cm to the bottom sample (14-15 cm), two species, *Globobulimina affinis* (d'Orbigny) and *Chilostomella oolina* (Schwager), are dominant, reaching maxima of 6-7 specimens per g in the 7-9-cm interval. The total number of living specimens per gram of $>63\text{-}\mu\text{m}$ sediment (Fig.

Table 1 Benthic foraminiferal data from OC-86/2-7-4

Species	Interval (cm)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15
<i>Chilostomella oolina</i>		-	1	-	-	-	1	6	29	26	20	9	4	5	2	2
<i>Cibicidoides kullenbergi</i>		6	1	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cibicidoides</i> spp.		3	1	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Globobulimina affinis</i>		-	-	1	-	-	2	12	33	29	10	5	3	5	1	1
<i>Hoeglundina elegans</i>		29	20	2	1	2	1	-	-	-	-	-	-	-	-	-
<i>Melonis barleeaanum</i>		2	12	30	18	17	5	-	-	-	1	-	-	-	-	-
<i>Melonis pompilioides</i>		-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oridorsalis tener</i>		10	5	1	-	-	-	-	-	-	-	-	-	-	1	-
<i>Parafissurina</i> sp.		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Planulina wuellerstorfi</i>		10	1	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pullenia simplex</i>		-	3	3	-	1	-	-	1	-	-	-	-	-	-	-
<i>Robertina bradyi</i>		2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Uvigerina</i> sp.		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown		-	-	-	1	-	-	-	2	-	1	-	-	-	-	-
Total no.		64	45	39	20	20	9	18	65	55	32	14	7	10	4	3
Weight, >63 µm fraction (g)		1.64	2.97	3.00	3.64	3.15	4.39	3.70	4.56	5.11	4.34	4.20	4.25	4.52	3.90	4.26

The number of specimens (>150 µm) of each species, the total number of specimens and the weight of the >63-µm fraction examined are shown for each sample interval.

2b) shows a maximum of 39 specimens per g in the 0-1-cm interval and a secondary maximum of 14 specimens per g at the 7-8-cm interval. The faunal data reveal that 73% of the living faunal assemblage was below 2 cm in OC-86/2-7-4. The presence of living benthic foraminifera at depth was verified by picking a few specimens of *G. affinis* from a wet subsample, dissolving the test in a 0.01 M EDTA solution and observing the stained protoplasm. The results from OC-86/2-7-4 differ from OC-86/2-5-3 data, which show that the majority of the living fauna (84%) are confined to the 0-1-cm interval. The number of living specimens per gram in the 0-1-cm interval is 13, with *Epistominella umbonifera* (Cushman) the dominant species, and 1 specimen per g in the 1-2-cm interval. Four specimens were found scattered within the 2-9-cm interval. The data from the two cores show that foraminiferal distributions within the sediments vary with water depth.

The foraminifera are vertically stratified in OC-86/2-7-4, with species-specific microhabitat preferences within the upper 15 cm of sediment. An infaunal habitat for calcareous benthic foraminifera within deep-sea sediments has not been noted previously, although it has been documented for some shallow-water calcareous species¹⁷⁻²⁴. Based on the OC-86/2 data, *M. barleeaanum*, *C. oolina* and *G. affinis* have infaunal microhabitat preferences. The species confined largely to the top 1 cm, *P. wuellerstorfi*, *C. kullenbergi* and *O. tener*, are more likely to have an epifaunal preference, although these species may be stratified within the 0-1-cm interval. A sampling interval of <1 cm is necessary to determine whether vertical stratification occurs within this interval. *H. elegans* is found throughout the top 2 cm, indicating that this species may have both an epifaunal and infaunal microhabitat, or that its distribution may be influenced by bioturbation moving living species downward from the surface. Bioturbation cannot be used, however, to explain the distribution of species below 2 cm, as *C. oolina* and *G. affinis* are not found in the 0-1-cm interval and these species and *M. barleeaanum* have subsurface maxima.

A correlation between benthic foraminiferal morphology and environment was noted by Bandy¹ in continental margin surface sediments; surface area and ornamentation of a number of species were shown to vary with water depth. Although several phenotype studies of benthic foraminifera have followed these initial observations, little is known about the functional adaptive morphology of the benthic foraminiferal test. The test morphologies of the benthic foraminifera in OC-86/2-7-4 show a distinct pattern with sediment depth (Fig. 1). Species found in the 0-2-cm interval, *H. elegans*, *P. wuellerstorfi*, *O. tener*, *C. kullenbergi* and *Cibicidoides* spp., have plano-convex or biconvex shapes, with wall pores found only on the spiral side or completely absent from the entire test. Of species found

living below 2 cm, *M. barleeaanum* has planispiral coiling with a rounded periphery, *C. oolina* is planispiral and cylinder shaped and *G. affinis* has triserially arranged chambers with a globular to ovate test. In contrast to the species found in the upper 2 cm, the dominant subsurface species have pores evenly distributed over most of the test.

The correlations between test shape, pore distribution and sediment depth suggest that these morphological variables are related to the microhabitat preference of the individual species. The plano-convex or biconvex shapes of the surface species may be advantageous for attachment to the bottom during times of bottom turbulence and for stability in traveling on or near the surface. For example, in shallow-water turbulent environments, *Cibicides* spp., which have plano-convex shapes, are attached to the substrate to resist transport or destruction in these high-energy regions. The plano-convex or biconvex shapes that would be advantageous for stability and mobility at or near the surface would be unnecessary within the sediment and these shapes are indeed absent.

Leutenegger and Hansen²⁵ noted that a number of species from low-oxygen (<0.3 ml l⁻¹ O₂) environments, including *Globobulimina pacifica*, a species closely related to *G. affinis*, had a lower density of mitochondria that were concentrated near pore openings within the cell, than specimens from higher-oxygen environments that had concentrations of mitochondria more evenly distributed throughout the cell. The concentration of mitochondria below the pore entrances was suggested to increase the diffusion of oxygen into the cell in low-oxygen environments. It was also found that an osmiophilic organic lining of *G. pacifica* did not cover the pore entrance, which was interpreted to allow a faster rate of molecular transport in low-oxygen environments. These observations support earlier conclusions¹⁶ that pores are important for gas exchange in foraminifera.

If gas-exchange morphological adaptations are significant, the distribution and density of pores should be distinctly different in epifaunal and infaunal species, because the microenvironments in which they live would be expected to have significantly different dissolved-oxygen levels. The infaunal species (*M. barleeaanum*, *C. oolina*, *G. affinis*) have pores evenly distributed over most of the test surface, which should be desirable for enhancing gas exchange in a low-oxygen environment. The pores found in *Cibicidoides* spp. and *P. wuellerstorfi* are found only on the spiral side and may be for protoplasmic streaming as well as for gas exchange, as these openings are much larger (~10 µm) than those found in other species (~1 µm). One species, *E. umbonifera*, which is found in the 0-1-cm interval of OC-86/2-5-3, is different from the other trochospiral species in that it has pores on both sides of the test. The foraminiferal test shapes

may reflect adaptations promoting gas exchange in low-oxygen environments within the sediment as well, as the shapes of species with subsurface maxima have higher surface area/volume ratios than the epifaunal species.

The distribution of live benthic foraminifera within these deep-sea sediments suggests that certain foraminifera with infaunal microhabitats are not directly controlled by overlying bottom-water conditions, but by physicochemical conditions within the sediment, as this environment is geochemically different from the sediment/water interface²⁶⁻²⁸. Some species do not appear to be confined to infaunal habitats, but instead are responding to a set of physicochemical variables independent of sediment depth. For example, *Chilostomella* and *Globobulimina* are found at depth in OC-86/2-7-4, but related species are common in California Borderland surface sediments (based on Rose Bengal stained data) associated with bottom water dissolved-oxygen values of $<3 \text{ ml l}^{-1}$ and generally $<1 \text{ ml l}^{-1}$ (ref. 29). The depth distribution of a particular species probably varies regionally in response to different physicochemical conditions within the sediments, ontogenetic differences in microhabitat requirements, or as a result of seasonal cycles in the deep sea.

Recent carbon-isotope data from pore waters in the upper few centimetres of Pacific Ocean and Atlantic Ocean sediments (ref. 30 and F. L. Sayles and W. B. Curry, in preparation) indicate that a $\delta^{13}\text{C}$ gradient of $\sim 1\%$ exists in the upper one to a few centimetres. The $\delta^{13}\text{C}$ values decrease with depth in the sediment because of the oxidation of ^{12}C -enriched organic matter, which results in a decrease in the $\delta^{13}\text{C}$ of the pore waters. The demonstration from the OC-86/2 data that a number of deep-sea benthic foraminifera do have infaunal microhabitats, together with the existence of $\delta^{13}\text{C}$ gradients within deep-sea sediments, indicate that the $\delta^{13}\text{C}$ disequilibrium of some benthic foraminifera can probably be explained in part by the $\delta^{13}\text{C}$ composition of the pore waters. Benthic foraminiferal carbon-isotope data^{10,11,13,31} show that plano-convex or biconvex forms, inferred to be epifaunal species, are generally nearest to isotope equilibrium, whereas species with shapes suggesting infaunal habitats are depleted in ^{13}C . For example, a species of *Globobulimina* was highly variable with $\delta^{13}\text{C}$ up to 2–3% below equilibrium of the overlying bottom water in the California Borderlands¹². One notable exception to this pattern, however, is *O. tener*, which has consistently lighter values than other biconvex forms. The existing data suggest that a relationship exists between carbon isotope composition and test morphology, which would be expected if the pore-water geochemistry of the microenvironment influences the benthic foraminiferal carbon-isotope data.

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1. Bandy, O. L. *21st Int. geol. Congr.*, Copenhagen, Pt 22, 7–25 (1960).
2. Barr, F. T. & Berggren, W. A. in *The Geology of Libya* (eds Salem, M. J. & Busirewil, M. T.) 163–192 (Academic, New York, 1981).
3. Berggren, W. A. & Aubert, J. *Geol. Surv. Prof. Pap.* 1213, 4–21 (1983).
4. Douglas, R. G. & Woodruff, F. in *The Sea Vol. 7* (ed. Emiliani, C.) 1233–1327 (Wiley-Interscience, New York, 1981).
5. Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* 3, 39–55 (1973).

6. Shackleton, N. J. & Opdyke, N. D. *Geol. Soc. Am. Mem.* 145 (eds Cline, R. M. & Hays, J. D.) 449–464 (1976).
7. Boyle, E. A. & Keigwin, L. D. *Jr Science* 218, 784–787 (1982).
8. Curry, W. B. & Lohmann, G. P. *Nature* 306, 577–580 (1983).
9. Shackleton, N. J., Imbrie, J. & Hall, M. A. *Earth planet. Sci. Lett.* 65, 233–244 (1983).
10. Woodruff, F., Savin, S. M. & Douglas, R. G. *Mar. Micropaleont.* 5, 3–11 (1980).
11. Belanger, P. E., Curry, W. B. & Matthews, R. K. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 33, 205–220 (1981).
12. Grossman, E. L., *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 47, 301–327 (1984).
13. Graham, D. W., Corliss, B. H., Bender, M. L. & Keigwin, L. D. *Jr Mar. Micropaleont.* 6, 483–497 (1981).
14. Savin, S. M. *et al. Mar. Micropaleont.* 6, 423–450 (1981).
15. Keigwin, L. D. *Jr Init. Rep. DSDP* 68, 445–453 (1982).
16. Boltovskoy, E. & Wright, R. *Recent Foraminifera*, 1–515 (Junk, The Hague, 1976).
17. Boltovskoy, E. *Contr. Cushman Fdn Foraminifera Res.* 17, 43–45 (1966).
18. Brooks, A. L. *Limnol. Oceanogr.* 12, 667–684 (1967).
19. Schafer, C. T. *Limnol. Oceanogr.* 16, 944–951 (1971).
20. Ellison, R. L. *Geol. Soc. Am. Mem.* 133, 247–262 (1972).
21. Matera, N. J. & Lee, J. J. *Mar. Biol.* 14, 89–103 (1972).
22. Frankel, L. *J. Paleont.* 46, 62–65 (1972).
23. Frankel, L. *J. Paleont.* 49, 563–565 (1975).
24. Ellison, R. L. & Peck, G. E., *J. Foramin. Res.* 13 (4), 231–241 (1983).
25. Leutenegger, S. & Hansen, H. J. *Mar. Biol.* 54, 11–16 (1979).
26. Froelich, P. N. *et al. Geochim. cosmochim. Acta* 43, 1075–1090 (1979).
27. Berner, R. R. in *The Environment of the Deep Sea* (eds Ernst, W. G. & Morin, J. G.) 154–176 (Prentice-Hall, Hemel Hempstead, 1982).
28. Sayles, F. L. *Geochim. cosmochim. Acta* 45, 1061–1086 (1981).
29. Douglas, R. G. & Heitman, H. L. *SEPM spec. Publ.* 27, 231–246 (1979).
30. McCorkle, D. C. & Emerson, S. R. *EOS* 64, 721 (1983).
31. Shackleton, N. J., Hall, M. A. & Boersma, A. *Init. Rep. DSDP* 74, 599–612 (1984).

Weak neutral currents and the origin of biomolecular chirality

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It has long been known that Earth's biochemistry is overwhelmingly dissymmetric or chiral^{1–4}. In model chemical systems^{5–7} that spontaneously evolve to a state dominated by either the L or the D enantiomer, parity violation in β -decay and that attributable to weak neutral currents (WNC) in molecules^{8,9} is thought to be too small to have any significant influence on the emergent chirality^{10,11}. Other conceivable systematic chiral influences are generally even weaker^{12–14}. We show here that there is a simple and extremely sensitive mechanism by which a minute but systematic chiral interaction, no stronger than the WNC interaction in amino acids, can, over a period of $\sim 15,000$ yr, determine which enantiomer will dominate. Such a mechanism is especially interesting when considering the origins of terrestrial biochemistry, particularly in view of the work by Mason and Tranter¹⁵, who found that it is the terrestrially dominant L amino acids that are favoured by the WNC interaction.

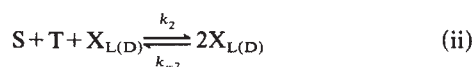
The process occurs in a randomly fluctuating environment. Chemical systems that, in conditions that are thermodynamically far from equilibrium, can evolve spontaneously to a chirally asymmetric state—that is 'break chiral symmetry'—exhibit a universal behaviour that is a consequence of the symmetry properties of the system^{14,16–18}. The amplitude α of the chiral dissymmetry, with the inclusion of fluctuations, obeys the stochastic (Langevin) equation

$$\frac{d\alpha}{dt} = -A\alpha^3 + B(\lambda - \lambda_c)\alpha + Cg + C'\eta f_2(t) + \varepsilon_1^{1/2}f_1(t) \quad (1)$$

in which A , B and C are constants that depend on the kinetics^{14,16}; $g = (\Delta E/kT)$, where k is the Boltzmann constant and T the temperature, is the factor by which the Arrhenius reaction rate constants for the L and the D enantiomers differ because of a small difference, ΔE , in their reaction barrier energies caused by an extremely weak chiral interaction¹⁴—such as WNC. In addition to such systematic effects, there is the fluctuating chiral influence from the environment, such as that attributable to circularly polarized ultraviolet light, represented by $C'\eta f_2(t)$, where $f_2(t)$ is assumed to be a normalized gaussian white noise. We estimate the numerical value of $C'\eta$ from the best known data on circularly polarized light. The intrinsic

thermodynamic fluctuations^{19,20} are represented by $\varepsilon_1^{1/2}f_1(t)$; again $f_1(t)$ is assumed to be a normalized gaussian white noise; $\varepsilon_1 = (Q/VN_A)$, where Q can be calculated from the chemical kinetics; V is the volume over which the concentration of the reactants may be assumed homogeneous and N_A is the Avogadro number. The most general stochastic equation must include fluctuations in A and B but, as they have no significant effect on the mechanism^{21,22}, we ignore them.

As an example and for later numerical considerations, we shall consider the following model scheme of reactions studied in detail in ref. 14



In this scheme, the chiral species X in the two enantiomeric forms X_L and X_D is produced from the achiral substrate S and T directly through reaction (i) and autocatalytically through reactions (ii). With a suitable supply of S and T (to maintain their concentrations at a fixed level), and the irreversible removal of X through reaction (iii), the system can be driven far from thermodynamic equilibrium. The variables of equation (1) for this system are: $\lambda \equiv [S][T]$ and $\alpha \equiv ([X_L] - [X_D])/2$ (where $[\]$ denote concentrations). When λ exceeds a certain critical value λ_c , the steady-state value of α switches from zero to either $\alpha > 0$ or $\alpha < 0$ (ref. 14). If the small chiral interaction influences reactions (i) and (ii) so that the rate constants of the L and D enantiomers are unequal, $k_{1L} = k_{1D}(1+g)$ and $k_{2L} = k_{2D}(1+g)$, then $C = [k_1 + k_2\beta_c]\lambda_c$, where $\beta = ([X_L] + [X_D])/2$, k_1 and k_2 are rate constants when $g = 0$, and the subscript 'c' denotes values at the critical point. Similar, but more involved, expressions may be obtained for A and B (ref. 14).

In contrast to the earlier studies²³⁻²⁵ that mainly examined such systems for $\lambda > \lambda_c$, we study the system as it slowly evolves through the critical point. The macroscopic steady states (when the fluctuations are ignored) and a sample fluctuating trajectory for the time evolution of α of equation (1) are shown in Fig. 1. When λ is well below λ_c , there is only one steady state, $\alpha \approx Cg/B(\lambda - \lambda_c) \ll 1$, which becomes unstable when λ goes beyond λ_c ; for $\lambda > \lambda_c$, the system has two new supercritical branches, $\alpha = \pm \sqrt{B(\lambda - \lambda_c)/A}$, to which it can evolve. In the absence of g , the two supercritical branches emerge symmetrically (as a parabola) from the point λ_c and, as the system evolves through the critical point, the fluctuations make both states equally probable. When $g \neq 0$ this is no longer true; one branch is favoured. The effect of g is most marked in the vicinity of the critical point where it separates the two stable supercritical branches by a minimum of $S = (3/2)(4/A)^{1/3}(Cg)^{1/3}$; in contrast, well above and below the critical point the shift is proportional to Cg . As $Cg \ll 1$, the fractional exponent indicates the enhanced sensitivity of the system near the critical point. However, in this region the α fluctuations are also large. Our aim is to obtain the probability of the system evolving to the favoured branch as λ goes through the critical point at a given rate.

An important factor in calculating this probability is $\varepsilon_1 = Q/VN_A$, which is derived assuming the system is homogeneous over the volume V . We estimate V by considering the homogenization that occurs through diffusion and, for prebiotic considerations, large-scale mixing as might occur in an ocean or a lagoon.

The evolution of the system may be considered in four stages (see Fig. 1). In stages I and IV, the system is well below and well above the critical point respectively; during stages II and III, it is in the vicinity of the critical point where the selection of branches takes place. In stages I and II, there is only one stable steady state and hence the system can be homogeneous over limitlessly large volume. In stage II, if $\alpha \ll 1$ and $\lambda \approx \lambda_c$, α

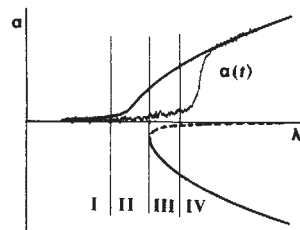


Fig. 1 A sample trajectory of α in equation (1) as λ increases through the critical point (fluctuations exaggerated). Solid line, stable steady states; dashed line, unstable steady state. Stages I-IV are explained in the text.

begins to grow slowly, essentially at an average rate Cg . In stage III, however, the system has two possible steady states and hence the volume over which it may be assumed homogeneous has an upper limit. In this stage, in a small volume δV , the autocatalysis of the chemistry (reflected in the term $B(\lambda - \lambda_c)$, $\lambda > \lambda_c$) will cause a fluctuation in α to grow; this growth, however, is curtailed by diffusion and large-scale mixing which transport the excess of α out of δV . As the rate of growth of α due to the chemical reactions is proportional to δV , whereas depletion is proportional to the surface area of δV , there is a 'nucleation volume' V_c below which α cannot grow. Within this volume V_c , homogeneity is maintained. Diffusion alone can maintain homogeneity over a length scale $l_D = \sqrt{D/B(\lambda - \lambda_c)}$, D being the diffusion coefficient^{26,27}. In our numerical simulation, in stage III, $B(\lambda - \lambda_c) \leq 10^{-11} \text{ s}^{-1}$, and with $D \sim 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ we see that $\lambda_D \sim 10^3 \text{ cm}$. Also, stages II and III are traversed in $\sim 6,000 \text{ yr}$, during which we may expect homogenization due to large-scale mixing to be on an oceanic scale. We assume this volume V_c to be at least $4 \times 10^9 \text{ l}$ ($1 \text{ km} \times 1 \text{ km} \times 4 \text{ m}$) for our estimate of ε_1 . As indicated by the fluctuating trajectory in Fig. 1, by the time stage IV is reached, if the system is already well within the region of attraction of the favoured (upper) branch, it becomes increasingly improbable for a fluctuation in α , occurring at least over a volume V_c , to be large enough to reach the unfavoured (lower) branch.

To obtain the probability for the selection of branches, we study the Fokker-Planck^{28,29} equation associated with equation (1), which describes the evolution of the probability density $P(\alpha, t)$ of α :

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial \alpha} (-A\alpha^3 + B(\lambda(t) - \lambda_c)\alpha + Cg)P(\alpha, t) + \left(\frac{\varepsilon}{2}\right) \frac{\partial^2}{\partial \alpha^2} P(\alpha, t) \quad (2)$$

where $\varepsilon = \varepsilon_1 + (C'\eta)^2$. We suppose, at $t = 0$, λ is well below the critical point and is gradually increasing. Fluctuations in λ have an insignificant role in the process of selection²¹, so we ignore them in our theoretical discussion, though not in our numerical simulation of the above model shown in Fig. 2. From equation (2) it follows that, for λ well below λ_c , $P(\alpha, t)$ is essentially a gaussian whose centre is at $\bar{\alpha} \approx Cg/B(\lambda_c - \lambda)$, very close to zero (stage I in Fig. 1). As λ increases at a reasonable rate ($\sim 10^{-5} \text{ M}^2 (10^4 \text{ yr})^{-1}$ for the model) in the vicinity of the critical point, $P(\alpha, t)$ is still a gaussian, but it begins to relax very slowly to the stationary distribution. Thus, in this stage (stages II and III in Fig. 1), $P(\alpha, t)$ is vanishingly small for large α and hence the $-A\alpha^3$ term may be neglected in comparison with the other terms.

For selection, then, we need only consider

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial \alpha} (B(\lambda(t) - \lambda_c)\alpha + Cg)P(\alpha, t) + \left(\frac{\varepsilon}{2}\right) \frac{\partial^2}{\partial \alpha^2} P(\alpha, t) \quad (3)$$

When $\lambda \approx \lambda_c$, the term $B(\lambda - \lambda_c)\alpha$, for $\alpha \ll 1$, also becomes small compared with Cg . Here we have a gaussian whose peak is shifting at a constant rate Cg , but whose width is also increasing

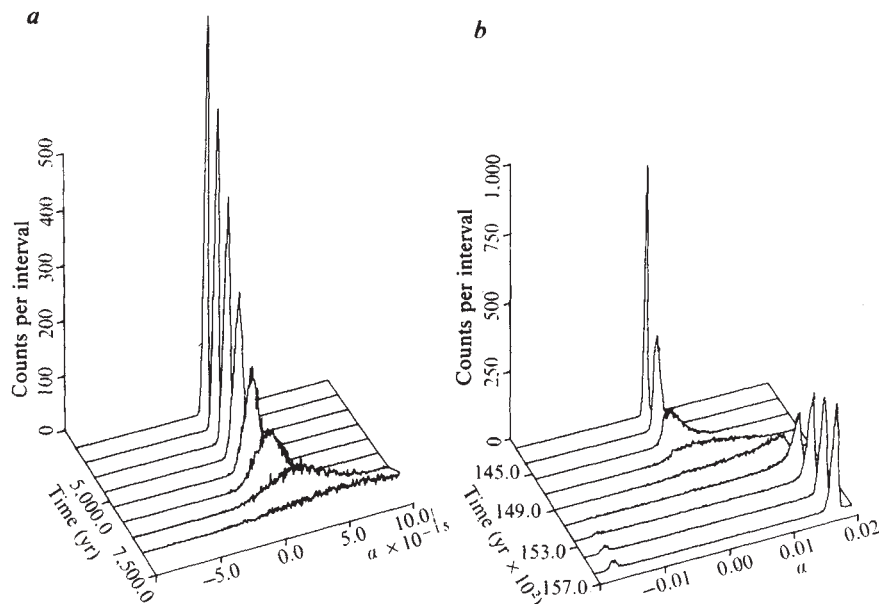


Fig. 2 Results of simulation of equation (2) by 5,000 sample trajectories of equation (1), with $\lambda(t) = \lambda_0 + \gamma t + 0.05 \lambda_c \times f(t)$, where $\lambda_0 = 0.8 \times 10^{-5} \text{ M}^2$, $\gamma = 3.171 \times 10^{-17} \text{ M}^2 \text{ s}^{-1}$, and $f(t)$ is normalized gaussian noise; t goes from 0 to 15,600 yr. Other parameters are as given in the text. Each curve is a histogram of the number of times $\alpha(t)$ fell within an interval $1/400$ of full α scale for the given t . a , $P(\alpha)$ drifting and spreading while λ is near λ_c , which here occurs at $t = 3,000$ yr. b , $P(\alpha)$ spreading and splitting for $\lambda \gg \lambda_c$. Note the change of scale in α by 10^{12} between a and b , because of which the number of trajectories per interval has sharply increased in b .

because of the 'diffusion term' containing ε . In a time interval T , the drift of the peak will be CgT , while the increase in the width will be $\sqrt{\varepsilon T}$. Thus, for sufficiently large T , even when $Cg \ll \sqrt{\varepsilon}$, CgT can exceed $\sqrt{\varepsilon T}$, which implies that much of $P(\alpha, t)$ will drift into the region $\alpha > 0$ (for $Cg > 0$). This is the heart of the selection mechanism. Now, as λ goes beyond λ_c (stage IV in Fig. 1), the term $B(\lambda - \lambda_c)$ is positive and will cause $P(\alpha, t)$ to spread out rapidly on either side. $P(\alpha, t)$, thus split into two parts, will accumulate around the macroscopic steady states because of the term $-A\alpha^3$; note, however, that by the time this term becomes important, how much of $P(\alpha, t)$ will have evolved into $\alpha > 0$ and $\alpha < 0$ regions will already have been determined. Thus, equation (3) is all we need to calculate the probability of branch selection—an approximation well supported by the numerical solution of the complete equation.

The solution to equation (3), well known^{29,30} for constant λ , can be extended to time-dependent λ . With $P(\alpha, 0) = \delta(\alpha - \alpha_0)$, the solution $P(\alpha, t|\alpha_0)$ is a gaussian that is drifting and spreading

$$P(\alpha, t|\alpha_0) = \frac{1}{[\pi z(t)]^{1/2}} \exp \left[-\frac{(\alpha - \bar{\alpha}(t))^2}{z(t)} \right] \quad (4)$$

where

$$\bar{\alpha} = \alpha_0 \exp(w(t)) + Cg \exp w(t) \int_0^t \exp(-w(t')) dt'$$

in which

$$w(t) = \int_0^t B(\lambda(t') - \lambda_c) dt'$$

and

$$z(t) = 2\varepsilon \int_0^t \exp(2 \int_{t'}^t B(\lambda(t'') - \lambda_c) dt'') dt'$$

For the probability of selection of the $\alpha > 0$ chiral steady state, P_+ , we have: $P_+ = \lim_{t \rightarrow \infty} \int_0^\infty P(\alpha, t|\alpha_0) d\alpha$. Because $P(\alpha, t|\alpha_0)$ is a gaussian and $\alpha_0 \approx 0$, we may write

$$P_+(t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{-x^2/2} dx \quad (5)$$

where

$$N = Cg \left[\exp w(t) \int_0^t \exp(-w(t')) dt' \right] / \sqrt{z(t)/2} \quad (6)$$

gives the number of standard deviations by which the peak of $P(\alpha, t)$ has drifted from the origin. Now, if we let $\lambda = \lambda_0 + \gamma t$ (λ_0 well below λ_c), for $t \rightarrow \infty$, we get

$$N = Cg(\varepsilon/2)^{-1/2} (B\gamma/\pi)^{-1/4} \quad (7)$$

Thus, equations (7) and (5) give the required probability for the selection of a branch resulting from a chiral interaction.

For the biomolecular context we consider the above model with kinetic constants $k_1 = 5 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$, $k_2 = 2.5 \times 10^{-5} \text{ M}^{-2} \text{ s}^{-1}$, $k_3 = 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, $k_{-1} = 2.5 \times 10^{-10} \text{ s}^{-1}$ and $k_{-2} = 1.25 \times 10^{-10} \text{ M}^{-1} \text{ s}^{-1}$. With these values the coefficients of equation (1) are: $A = 1.7 \times 10^{-7} \text{ M}^{-2} \text{ s}^{-1}$, $B = 2.5 \times 10^{-5} \text{ M}^{-2} \text{ s}^{-1}$, $C = 2.5 \times 10^{-10} \text{ M s}^{-1}$ and $\lambda_c = 1.0 \times 10^{-5} \text{ M}^2$; all concentrations are $\sim 10^{-3} \text{ M}$ or less during the process of selection and $[X] \sim 10^{-2} \text{ M}$ well above λ_c . If we suppose $[S]$ and $[T]$ are increasing slowly so that $\lambda = [S][T]$ increases from $0.5 \lambda_c$ to $1.5 \lambda_c$ in 10,000 yr, then $\gamma = 3.2 \times 10^{-17} \text{ M}^2 \text{ s}^{-1}$. For $\varepsilon_1 = Q/VN_A$, $Q = (k_1 \lambda_c)/2$ for the model¹⁴, and we take $V = 4 \times 10^9 \text{ l}$ as explained above, so that $\varepsilon_1 \approx 10^{-43} \text{ M}^2 \text{ s}^{-1}$. Using the results of Mason and Tranter for WNC¹⁵, we take $g = 10^{-17}$, which makes $Cg = 2.5 \times 10^{-27}$. For the magnitude of the random chiral influences, we take $C'\eta = 3 \times 10^{-22}$, an estimate explained below. Using these values in equation (7) we get $N \approx 2.0$, which implies $P_+ \approx 0.98$, a 98% chance that the enantiomer favoured by WNC will emerge dominant even though the r.m.s. values of the random chiral influences are five orders of magnitude larger. Such sensitivity cannot be realized if the system does not evolve through the critical point.

To check the validity of our approximation of using equation (3) instead of equation (2), we numerically modelled the stochastic equation (1), with $\lambda = \lambda_0 + \gamma t + 0.05 \lambda_c f(t)$, $f(t)$ being a gaussian noise. The results are shown in Fig. 2. For easier graphical visualization, we set $\varepsilon = 3.9 \times 10^{-43}$. Then, according to equation (5), $N = \sqrt{2}$, implying $P_+ = 0.921$ selectivity. Figure 2 is the result of averaging 5,000 trajectories and gives a $P_+ = 0.916$ in good agreement with the analytical result. If λ evolves extremely slowly, the approximation of using equation (3) is not valid. In the limit of infinitely slow λ_D , the results in refs 14, 16 can be used; for the intermediate region no analytical results exist.

For the estimate of the r.m.s. value of the random environmental factors, we take circularly polarized light in the ultraviolet range to be typical. (Hardly any reliable data exist for other effects.) Chirally selective photochemical effects of circularly polarized light are well known³¹⁻³³. In our model, let us assume that chirally selective degradation of X (back reaction of reaction

(i)), at a rate $k[X]$, occurs with a mean life of about 3,000 yr, that is $k' \approx 10^{-11} \text{ s}^{-1}$ for the average solar intensity. (Note, this is also the racemization rate.) For 100% circularly polarized light, the rate of decay of one of the enantiomers is faster by a factor $s \approx 10^{-3}$ or less for most molecules^{32,33}, although there are exceptions³⁴. Only a fraction $q \approx 10^{-3}$ of the solar intensity at dawn and dusk is found to be circularly polarized in the infrared frequencies and is at least an order of magnitude smaller for the ultraviolet³⁵. The sense of polarization depends on the direction and on the average it is zero. Considering the reduction of q for daylight intensities and attenuating factors in a large body of water, we may take $q_{rms} \approx 10^{-6}$ with a correlation time $\tau \approx 10^3 \text{ s}$. On the evolutionary timescale of 10^{10} – 10^{12} s considered here, this may be considered white noise of strength $\sqrt{2\tau} q_{rms}$

(equivalent to diffusion constant of $(1/2\tau)$ steps per unit time, each of length $2\tau q_{rms}$). Thus, the rate of asymmetric synthesis is $k[X_{L(D)}]qs$; when q is a fluctuating quantity it may be represented by a gaussian white-noise term $C'\eta f_2(t)$, with $C'\eta = k'([X_L] + [X_D])/2s\sqrt{2\tau} q_{rms}$. By analogy with Cg, we may define $C' = k'([X_L] + [X_D])/2$ and $\eta = s\sqrt{2\tau} q_{rms}$. With the above numerical values and $[X] \approx 10^{-3} \text{ M}$, $C'\eta \approx 3 \times 10^{-22}$, the value used in our numerical estimates.

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- Mason, S. F. *Int. Rev. phys. Chem.* **3**, 217–241 (1983); *Nature* **311**, 19–23 (1984).
- Elias, W. E. *J. chem. Educ.* **49**, 448–454 (1972).
- Miller, S. W. & Orgel, L. E. *The Origin of Life on Earth* (Prentice-Hall, New Jersey, 1974).
- Fox, S. W. & Dose, K. *Molecular Evolution and the Origin of Life* (Dekker, New York, 1977).
- Frank, F. C. *Biochim. biophys. Acta* **11**, 459–463 (1953).
- Seelig, F. F. *J. theor. Biol.* **31**, 335–361 (1971).
- Decker, P. *J. molec. Evol.* **4**, 49–65 (1974).
- Zel'dovich, Ya. B., Saakyan, D. B. & Sobel'man, I. I. *Pis'ma Zh. eksp. teor. Fiz.* **25**, 106–109 (1977) (*J. exp. theor. phys. Lett.* **25**, 94–98).
- Hegstrom, R. A., Rein, D. W. & Sanders, P. G. *H. J. chem. Phys.* **73**, 2329–41 (1980).
- Thiemann, W. (ed.) *Origins Life* **11**, 1–194 (1981).
- Keszthelyi, L. *Origins Life* **14**, 375–382 (1984).
- Mead, C. A. & Moscovitz, A. *J. Am. chem. Soc.* **102**, 7301–7302 (1980).
- Peres, A. *J. Am. chem. Soc.* **102**, 7389–7390 (1980).
- Kondepudi, D. K. & Nelson, G. W. *Physica* **125A**, 465–496 (1984).
- Mason, S. F. & Tranter, G. E. *JCS chem. Commun.*, 117–119 (1983); *Molec. Phys.* **53**, 1091–1111 (1984).
- Kondepudi, D. K. & Nelson, G. W. *Phys. Rev. Lett.* **50**, 1023–1026 (1983).
- Kondepudi, D. K. & Prigogine, I. *Physica* **107A**, 1 (1981).
- Sattinger, D. H. *Lecture Notes in Mathematics* No. 762 (Springer, Berlin, 1979).
- Mangel, M. *J. chem. Phys.* **69**, 3697–3708 (1978).
- Keizer, J. *J. chem. Phys.* **69**, 2609–2620 (1978).
- Kondepudi, D. K. in *Fluctuations and Sensitivity in Nonequilibrium Systems* (eds Horthemke, W. & Kondepudi, D. K.) 204–213 (Springer, Berlin 1984).
- Kondepudi, D. K. & Nelson, G. W. *Phys. Lett.* **106A**, 203–206 (1984).
- Mangel, M. *Phys. Rev. A* **24**, 3226–3238 (1981).
- Morozov, L. L., Kuz'min, V. V. & Gol'danskii, V. I. *Origins of Life* **13**, 69–101 (1983).
- Morozov, L. L., Kuz'min, V. V. & Gol'danskii, V. I. *Pis'ma Zh. eksp. teor. Fiz.* **39**, 344–345 (1984); *JETP Lett.* **39**, 414–416 (1984).
- Nicolis, G. & Prigogine, I. *Self Organization in Nonequilibrium Systems* (Wiley, New York, 1977).
- Nicolis, G. & Malek-Mansour, M. *Phys. Rev. A* **29**, 2845–2853 (1984).
- Nitzan, A., Ortoleva, P., Deutch, J. & Ross, J. *J. chem. Phys.* **61**, 1056–1074 (1974).
- Van Kampen, N. G. *Stochastic Processes in Physics and Chemistry* (North-Holland, Amsterdam, 1981).
- Uhlenbeck, G. E. & Ornstein, L. S. *Phys. Rev.* **36**, 824–841 (1930).
- Kuhn, W. & Braun, F. *Naturwissenschaften* **17**, 227–228 (1929).
- Kagan, H. B. *et al Tetrahedron Lett.* **27**, 2479–2482 (1971).
- Kagan, H. B., Balavoine, G. & Moradpour, A. *J. molec. Evol.* **4**, 41–48 (1974).
- Flores, J. J., Bonner, W. A. & Massey, G. A. *J. Am. chem. Soc.* **99**, 3622–3624 (1977).
- Angel, J. R. P., Illing, R. & Martin, P. G. *Nature* **238**, 389–390 (1972).

Presynaptic neurones may contribute a unique glycoprotein to the extracellular matrix at the synapse

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As the extracellular matrix at the original site of a neuromuscular junction seems to play a major part in the specificity of synaptic regeneration^{1–5}, considerable attention has been paid to unique molecules localized to this region^{6–11}. Here we describe an extracellular matrix glycoprotein of the elasmobranch electric organ that is localized near the nerve endings. By immunological criteria, it is synthesized in the cell bodies, transported down the axons and is related to a glycoprotein in the synaptic vesicles of the neurones that innervate the electric organ. It is apparently specific for these neurones, as it cannot be detected elsewhere in the nervous system of the fish. Therefore, neurones seem to contribute unique extracellular matrix glycoproteins to the synaptic region. Synaptic vesicles could be involved in transporting these glycoproteins to or from the nerve terminal surface.

Cholinergic synaptic vesicles have been purified to near homogeneity from the electric organs of elasmobranchs^{12,13} and have been used to generate nerve-terminal-specific antiserum^{14,15}. Using synaptic vesicles purified from the electric organ of *Discopyge ommata* as antigen, we have generated monoclonal antibodies to unique vesicle components copurifying with synaptic vesicle contents during size fractionation. The vesicle-specific monoclonal antibodies define at least four different antigenic determinants (SV1–SV4). The SV1 region is part of the same proteoglycan-like molecule that binds monoclonal antibody tor70 (ref. 16). The other three sites have not yet been described. SV2 is fully accessible to antibody in intact vesicles, whereas the rest become accessible only after sonication

or detergent treatment, and so are presumably exposed on the inside of the vesicle¹⁷.

The relative concentration of each antigenic determinant was measured by quantitative immunoblotting of synaptic vesicles (Table 1). All four antigens are present in electric organ synaptosomes at about one-tenth of the concentration in vesicles, which is as expected because acetylcholine and ATP in the vesicles have ~10 times higher concentrations per mg protein¹² than in synaptosomes¹⁸. When the relative concentrations of all four determinants were measured on homogenates of electric organ (Table 1), SV2 and SV3 had low specific activities, as expected from earlier calculations¹². The specific activity in the electric organ of the SV4 antigenic sites, however, was ~10 times higher than expected, suggesting that ~90% of it is not in synaptic vesicles. The SV4 site, unlike the other three, is found in high concentrations in an extracellular matrix fraction of the electric organ, where the specific activity is higher than in synaptic vesicles (Table 1). As 77% of the total SV4 (but only 2.1% of the total protein) is recovered in the extracellular matrix, most of the SV4 antigen in electric organ may not be in synaptic vesicles, but in the matrix.

The molecules carrying SV4 and SV1 are highly restricted in their location, whereas the SV2 and SV3 antigens occur in all nerve terminals of the electric fish. In homogenates of brain and spinal chord SV4 and SV1 were less than 2% of the value predicted from the amount of SV2 and equal to background levels measured using liver homogenates. By immunofluorescence the cell bodies of the electromotor nucleus contain large amounts of the SV2, SV1 and SV4 sites, whereas synapses in other brain regions have SV2 but no detectable SV4 or SV1 (K. Buckley, unpublished observations), hence the SV4 and SV1 antigens are restricted to electromotor nucleus neurones.

The molecule in synaptic vesicles carrying the SV4 site has a heterogeneous mobility and its antigenicity is destroyed by exposure to conditions¹⁹ that remove carbohydrate side chains from proteins (Fig. 1). The molecule thus has the properties of a glycosylated protein whose antigenicity is associated with oligosaccharides. The SV4 antigen from the extracellular matrix fraction shows the same heterogeneous electrophoretic mobility, and the same susceptibility to mild proteolysis and sensitivity

Table 1 Relative amounts of unique antigenic determinants

	SV4	SV1	SV2	SV3
	(c.p.m. per μg protein) $\times 10^{-2}$			
Synaptic vesicles	111 $\pm$ 4	366 $\pm$ 12	170 $\pm$ 2	87 $\pm$ 2
Synaptosomes	17.4 $\pm$ 0.4	35 $\pm$ 1	14.8 $\pm$ 0.5	6.7 $\pm$ 0.5
Extracellular matrix	158 $\pm$ 3	18.4 $\pm$ 0.7	2.1 $\pm$ 0.9	1.2 $\pm$ 0.8
Electric organ homogenate	4.4 $\pm$ 0.4	3.8 $\pm$ 0.5	0.54 $\pm$ 0.1	0.32 $\pm$ 0.08

The concentration of antigenic sites was determined by an indirect solid-phase radioimmunoassay¹⁶. Samples were solubilized in 0.2 M NaCl, 10 mM HEPES, 1% SDS pH 7.0 and spotted on nitrocellulose filters. After saturating nonspecific sites with carrier protein (5% bovine serum albumin) monoclonal antibody was added in excess (1/100 dilution of hybridoma supernatants). Bound antibody was detected with affinity-purified iodinated goat anti-mouse IgG. The region where binding was linear with protein concentration was used to calculate antigen concentration by linear regression, $\pm$ s.e.m. Although the data from only one experiment are given, the relative ratios of antigen concentration have been confirmed at least four times. The extracellular matrix fraction was purified from the electric organ of *D. ommata* by the procedure of McMahan and his colleagues¹¹, except that detergent extraction was carried out at pH 10.5 to remove adventitiously adsorbed protein.

to deglycosylation as its vesicle counterpart, although the matrix-derived material seems to have an even slower electrophoretic mobility on polyacrylamide gels. Thus, the vesicle and extracellular matrix forms of the antigen are similar, but not identical.

Molecules bearing the SV4 antigen must form a tight association in the extracellular matrix fraction, as <10% is released by detergent extraction during matrix isolation. In contrast, >82% of the synaptic vesicle form is solubilized under identical conditions. The SV4 antigen can be solubilized completely from the extracellular matrix by high salt extraction (4M guanidinium chloride). Treatment of sonicated synaptic vesicles with either high salt or pH 11 buffer in the absence of detergents solubilizes <2% of the SV4 antigenic site, suggesting that it is part of an integral membrane protein.

Localization of the antigenic material in the synaptic extracellular matrix was confirmed by a combination of immunoelectron microscopy and biochemical data. Buckley *et al.*²⁰ have described an antibody to synaptic vesicles (tor70) which also binds to the outside of nerve terminals in the region where they face the electrocytes. SV1, SV3 and SV4 sites are also restricted to this extracellular domain (K. Buckley unpublished observations). To examine extracellular binding sites, we gently sheared electric organ to give large sheets of membrane covered with intact terminals^{21,22}.

To demonstrate that the SV4 antigenic sites in the extracellular matrix fraction correspond to those seen in the synaptic cleft in immunoelectron microscopy studies, antibodies were bound to the intact nerve terminals as before, except that ¹²⁵I-labelled goat anti-mouse second antibodies were used instead of fluorescent or peroxidase-labelled ones. With antibodies to the intravesicular antigens (SV1, SV3, SV4), the sheets became heavily labelled. When such sheets, labelled indirectly with antibodies to the SV4 antigen, were subjected to the standard fractionation procedure, 75% of the radioactivity occurred in the extracellular matrix fraction. In contrast, 3% of SV3 and 11% of SV1 were recovered in that fraction. (In this experiment the extracting buffer was at pH 7.5, not 10.5, to avoid dissociating antigen/antibody complexes.)

The SV4 antigenic site seems to be generated in the neuronal cell bodies; all four antigenic determinants could be measured in the axons and in the electromotor nucleus. To investigate whether these antigenic sites are generated in the periphery, the axons from this nucleus to the electric organ were cut. Three weeks later, the relative concentrations of the SV4 antigenic site in the electromotor nucleus had increased slightly (Table 2), suggesting that it is not derived by retrograde transport from the nerve terminals. Although SV2 and SV3 increased to the same extent, SV1 fell to 17% of its original concentration. To

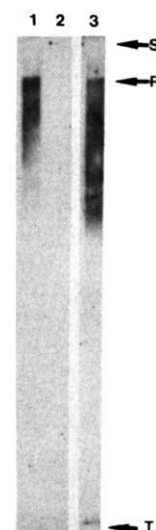


Fig. 1 SV4 antigen exists on a glycoprotein. Synaptic vesicle proteins were deglycosylated by an acid hydrolysis procedure that specifically cleaves polysaccharide chains from glycoproteins¹⁹. Proteolytic digestion was performed for 5 min at 4 °C in the presence of 0.5% Nonidet P-40 with a twofold excess of trypsin over vesicle protein and was interrupted by boiling the sample in final sample buffer. Equivalent amounts of synaptic vesicle protein were run on an 8 M urea/8% SDS polyacrylamide gel and then transferred to nitrocellulose²⁷. The nitrocellulose filters were then exposed to monoclonal antibody (1/100 dilution) and subsequently to ¹²⁵I-goat anti-mouse antiserum using standard washing procedures. Filters were then dried and exposed to Kodak X film with an intensifying screen for 15 h at -80 °C. Lane 1, untreated synaptic vesicles (2 μg protein); lane 2, deglycosylated synaptic vesicles (5 μg protein); lane 3, trypsin-digested synaptic vesicle proteins (2 μg vesicle protein, 4 μg trypsin). S, start of stacking gel; R, start of running gel; T, trypsin present in sufficiently high quantities to bind goat anti-mouse antibodies nonspecifically. The deglycosylation procedure¹⁹ increased the electrophoretic mobility of a glycoprotein standard ovomucoid and did not destroy the antigenicity of the SV2 antigen but increased its relative molecular mass (data not shown).

show that the antigenic sites generated in the electromotor nucleus are transported to the nerve terminals, the nerves from the electromotor nucleus to the electric organ were crushed and the accumulation of antigens at the crush measured. SV1 accumulated only distally, implying that the SV1 antigenic site is probably generated at the nerve terminal (Table 2). SV4, on the other hand, increased three- to fourfold on the proximal side of the crush, implying anterograde transport, and also increased on the distal side, indicating retrograde transport.

As anterograde transport has been associated only with movement of newly synthesized material from the perikaryon²³, we propose that, unlike SV1, the SV4 antigen is made in the cell body. Direct support for a presynaptic origin of the SV4 antigen was obtained by injecting ³⁵S-methionine into the right lobe of the electromotor nucleus. Although the total concentration of label in the ipsi- and contralateral electric organs was indistinguishable (presumably because of systemic leakage of ³⁵S-methionine), immunoadsorption with anti-SV4 specifically precipitated 10–20% of the labelled protein from an extracellular matrix fraction of the right (injected) electric organ. In contrast, only 0–5% was precipitated from the contralateral extracellular matrix. Immunoprecipitation with a monoclonal antibody directed against a non-neural antigen showed no difference between the right and left sides. We therefore conclude that the SV4 antigen is synthesized in the electromotor nucleus and transported to the electric organ, and that the material that accumulated distal to a crush represents antigen that is recycling to the cell body from the nerve terminal. Analogous retrograde transport of vesicle proteins has been observed in vertebrate neurones^{24–26} and has been interpreted as the reversal of anterograde flow at the nerve terminal²³.

Table 2 Demonstration that SV4 antigen is made in the cell body

Antigenic site/fraction	SV4	SV1 (c.p.m. per μg protein) $\times 10^{-2}$	SV2	SV3
Electromotor nucleus	1.8 $\pm$ 0.1	0.83 $\pm$ 0.081	2.01 $\pm$ 0.2	0.6 $\pm$ 0.1
Electromotor nucleus after denervation	2.8 $\pm$ 0.2	0.14 $\pm$ 0.01	3.0 $\pm$ 0.3	1.00 $\pm$ 0.2
Nerve	0.33 $\pm$ 0.03	0.067 $\pm$ 0.009	0.026 $\pm$ 0.008	0.013 $\pm$ 0.009
Nerve proximal to crush	1.5 $\pm$ 0.2	0.09 $\pm$ 0.02	0.10 $\pm$ 0.01	0.05 $\pm$ 0.01
Nerve distal to crush	1.87 $\pm$ 0.06	0.52 $\pm$ 0.10	0.15 $\pm$ 0.02	0.06 $\pm$ 0.01

Conditions for operating on electric fish and denervating the electric organ have been described previously²². All the nerves to one of the two electric organs were cut and the animal was killed 3 weeks after surgery. The experimental and the control electromotor nuclei were removed separately. In axonal transport experiments, sections of nerves to the electric organ were crushed just proximal to their entry into the electric organ. Three days later the fish were killed and 2–4-mm segments proximal and distal to the crush removed. Samples were prepared by homogenization of tissue in 1% SDS buffer as before, and the antigen concentration measured by dot blotting. Specificity of antibody binding was verified by comparison with synaptic vesicle samples on Western blots.

Two more antigenic determinants shared by the synaptic vesicles and the extracellular matrix have been identified by raising monoclonal antibodies to electric organ extracellular matrix. Twenty-five of these bind to innervated sheets of electric organ membrane in a pattern indistinguishable from that obtained by Buckley *et al.*²⁰ with specific anti-synaptic vesicle antiserum. Two of these also bind to synaptic vesicle components that co-purify with vesicle contents on permeation chromatography. Both also block binding of ¹²⁵I-labelled antibody to the SV4 antigenic site on synaptic vesicles and on extracellular matrix, and have Western blot patterns on synaptic vesicle proteins similar to those in Fig. 1. Hence, all three antigenic sites are probably on the same molecule and physically close to each other but are not identical to SV4. The extracellular matrix (ECM)1 antigenic site closely resembled SV4 except that the ratio of extracellular matrix to vesicle binding sites differed by a factor of 2.5. Nerve crush experiments showed that, like SV1, ECM2 was only transported retrogradely and so is also probably generated at the nerve terminal. ECM1 and ECM2 are found only in the cell bodies and at the nerve terminals of the neurones innervating the electric organ. Only one synaptic vesicle component has been found in the extracellular matrix by immunological criteria; the extracellular matrix component is the only one found in vesicles. We conclude that three monoclonal antibodies to different but spatially contiguous antigenic sites bind to a glycoprotein of the synaptic extracellular matrix. The glycoprotein originates in the presynaptic neurone and is modified at the nerve terminal region.

Although the synaptic cleft region of the neuromuscular junction contains unique antibody and lectin-binding sites^{6–8}, the extracellular matrix glycoprotein recognized by these antibodies may be the first shown to be transported down the axon to the nerve terminals. Using the nerve cell to synthesize extracellular matrix precursors is an attractive solution to the problem of how to create synapse-specific domains in the extracellular matrix.

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1. Marshall, L. M., Sanes, J. R. & McMahan, U. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3073–3077 (1977).
2. Sanes, J. R., Marshall, L. M. & McMahan, U. J. *J. Cell Biol.* **83**, 357–370 (1978).
3. Burden, S. J., Sargent, P. B. & McMahan, U. J. *J. Cell Biol.* **82**, 412–425 (1979).

4. Sanes, J. R. *A. Rev. Physiol.* **45**, 581–601 (1983).
5. McMahan, U. J., Slater, C. R. & Marshall, R. M. *J. Cell Biol.* **98**, 1453–1473 (1984).
6. Sanes, J. R. & Hall, Z. W. *J. Cell Biol.* **83**, 357–370 (1979).
7. Burden, S. J. in *Monoclonal Antibodies to Neural Antigens* (eds McKay, R., Raff, M. C. & Reichardt, L. F.) 247–257 (Cold Spring Harbor Laboratory, New York, 1981).
8. Sanes, J. R. & Cheney, J. M. *Nature* **300**, 646–648 (1982).
9. Sanes, J. R. *J. Cell Biol.* **93**, 442–452 (1982).
10. Anderson, M. J. & Fambrough, D. M. *J. Cell Biol.* **97**, 1396–1411 (1983).
11. Godfrey, E. W., Nitkin, R. M., Wallace, B. G., Rubin, L. L. & McMahan, U. J. *J. Cell Biol.* **99**, 615–627 (1984).
12. Carlson, S. S., Wagner, J. A. & Kelly, R. B. *Biochemistry* **17**, 1188–1199 (1978).
13. Tashiro, T. & Stadler, H. *Eur. J. Biochem.* **90**, 479–487 (1978).
14. Sanes, J. R., Carlson, S. S., von Wedel, R. J. & Kelly, R. B. *Nature* **280**, 403–404 (1979).
15. Carlson, S. S. & Kelly, R. B. *J. Cell Biol.* **87**, 98–103 (1980).
16. Carlson, S. S. & Kelly, R. B. *J. Biol. Chem.* **258**, 11082–11091 (1983).
17. Buckley, K. M. & Kelly, R. B. *J. Cell Biol.* (submitted).
18. Morel, N., Israel, M., Manaranche, R. & Mastour-Frachon, P. *J. Cell Biol.* **75**, 43–55 (1975).
19. Edge, A. S. B., Faltyeuck, C. R., Hof, L., Reichert, L. E. Jr & Weber, P. *Analyt. Biochem.* **118**, 131–137 (1981).
20. Buckley, K. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7342–7346 (1983).
21. Morel, N., Manaranche, R. & Israel, M. *J. Physiol., Paris* **78**, 433–442 (1982).
22. Hooper, J. E., Deutsch, J. W., Miljanich, G. P., Brasier, A. R. & Kelly, R. B. *J. Physiol., Paris* **78**, 443–453 (1982).
23. Grafstein, B. & Forman, D. S. *Physiol. Rev.* **60**, 1167–1283 (1980).
24. Holtzman, E. *Neuroscience* **2**, 327–355 (1977).
25. Brimjoin, S. & Helland, L. *Brain Res.* **102**, 217–228 (1976).
26. Broadwell, R. D. & Brightman, M. W. *J. comp. Neurol.* **185**, 31–74 (1979).
27. Burnette, W. N. *Analyt. Biochem.* **112**, 195–203 (1981).

Carbon-dioxide-induced exocytotic insertion of H⁺ pumps in turtle-bladder luminal membrane: role of cell pH and calcium

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The contents of endocytic vesicles and other intracellular organelles (such as Golgi and microsomes) are acidified by an electrogenic proton-translocating ATPase that is remarkably similar to that found in urinary epithelia^{1–10}. We recently found that the number of H⁺ ATPases in the apical plasma membrane of these epithelia is regulated by exocytotic insertion of endocytic vesicles whose membranes contain this H⁺ pump^{2,10}. Carbon dioxide, a major stimulus for urinary acidification, causes rapid fusion of these vesicles with the luminal membrane, thereby inserting these pumps there and increasing the rate of net transepithelial H⁺ secretion; CO₂ also inhibits endocytic retrieval of the pumps from the luminal membrane¹¹. Such reciprocal regulation of endocytosis and exocytosis by a physiological modulator makes this system particularly attractive for studying the cellular events regulating membrane fusion. Here we present evidence that CO₂ induces exocytosis by a cascade of events, the first step of which is cytoplasmic acidification. Cell acidification then increases calcium activity, which causes the fusion event.

The epithelial layer of the turtle urinary bladder is comprised of two cell types, one of which, the mitochondrion-rich cell, is enriched in carbonic anhydrase and in vesicles containing proton pumps; hence, it is thought to be the cell responsible for transepithelial H⁺ transport^{2,12}. When these bladders are mounted in Ussing chambers, H⁺ secretion can be measured as the short-circuit current obtained when sodium transport is inhibited by ouabain¹². To investigate whether CO₂ causes exocytotic insertion of H⁺ pumps by acidifying the cytoplasm, we added 5 mM butyrate or acetate to the luminal medium and found that they stimulated the H⁺ current in these bladders and that their effect became greater as the pH of the medium was reduced. At pH 7, butyrate increased the H⁺ current by 33%, at pH 6 the H⁺ current increased by 116%, whereas at pH 5 it increased by 179% (*n* = 7 experiments). Quantitatively similar results were obtained with acetate. Because the *pK* of butyrate and acetate is ~4.8 and the undissociated acid is more permeant, these results suggest that the species of the molecule that is

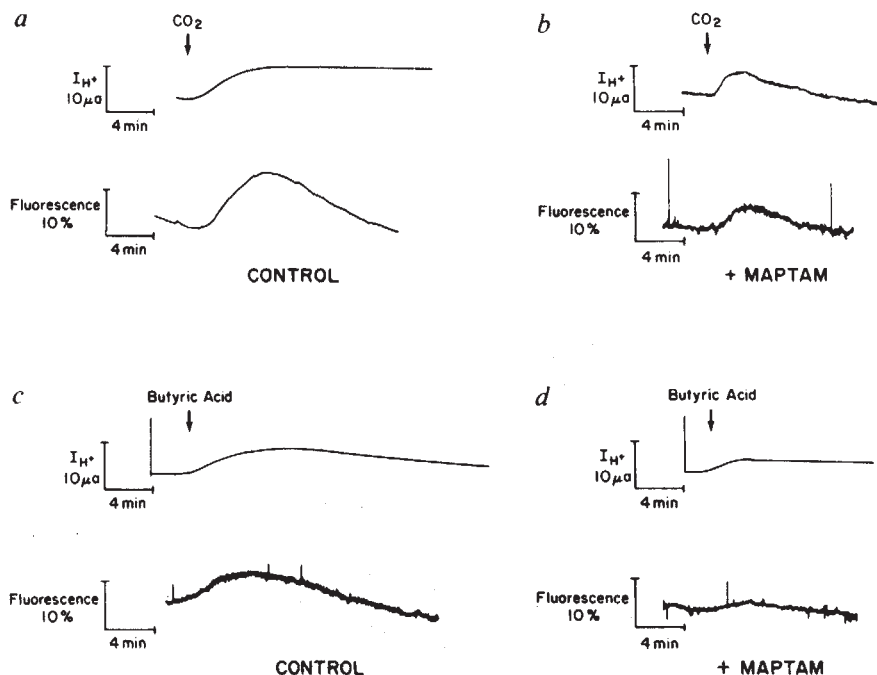


Fig. 1 Exocytosis of fluorescent dextran simultaneously with an increase in H^+ secretion in the turtle urinary bladder. The bladders were perfused on both sides by continuously circulating freshly aerated Ringer's solution with a peristaltic pump. The bladder was initially exposed on the luminal side for 1 h with a 5% CO_2 Ringer's (pH 7.5) with FITC dextran (relative molecular mass 70,000) dissolved at 100 mg ml^{-1} followed by 1 h of CO_2 -free FITC dextran Ringer's. The luminal solution was then changed to CO_2 -free Ringer's without FITC dextran pH 6.0 and was passed through a flow cell in a ratio fluorometer (Farrand Optical). After 1 h, both the H^+ current and the fluorescence fell to a low steady-state level. The luminal perfusate was then changed to one containing 5 mM butyric acid, pH 6.0 (a, b), causing a broad peak in the fluorescence tracing and an increase in the H^+ secretion. The solution was then changed to 5% CO_2 Ringer's pH 6.0 (c, d) and a similar rise in H^+ secretion and release of FITC dextran was found. b, d Show the effect of pretreatment of the luminal side of the bladders with 0.1 mM MAPTAM (Calbiochem) for 1 h.

Table 1 Effect of colchicine (a) and MAPTAM (b) on H^+ current in paired turtle hemibladders

a	Control	Colchicine-treated
Butyrate ($n = 7$)	24 ± 6	$13 \pm 4^*$
Acetate ($n = 7$)	10 ± 3	$4 \pm 4^*$
b	Control	MAPTAM-treated
CO_2 ($n = 5$)	11.8 ± 4.6	$1.7 \pm 5^*$
Butyrate ($n = 8$)	11.2 ± 5	$2.9 \pm 1.7^*$
Acetate ($n = 3$)	3.0 ± 0.8	$0.8 \pm 0.6^*$

Paired hemibladders from freshwater turtles were mounted in Ussing chambers, bathed with CO_2 -free Ringer's solution and continuously short-circuited using an automatic voltage clamp. The media were circulated by a gas-lift device using CO_2 -free air. Ouabain (0.5 mM) was added to the serosal side in all experiments. The pH of the luminal solution was 6.0. The composition of the CO_2 -free Ringer's solution was 85 mM NaCl, 3.5 mM KCl, 0.3 mM NaH_2PO_4 , 1.65 mM Na_2HPO_4 , 0.5 mM $MgSO_4$, 1 mM $CaCl_2$. a, Colchicine (0.1 mM) was added to one member of a pair and 2 h later 5 mM Na-butyrate or Na-acetate was added to the luminal side. The peak increase in the H^+ current is given. Current is given as μA per $8 \text{ cm}^2 \pm \text{s.d.}$ b, MAPTAM (50 μM dissolved in dimethyl sulphoxide) was added to one member of a pair whereas the other received diluent only. Two hours later, CO_2 was added isohydrically to both media. In other experiments, butyrate or acetate (5 mM each) was added to the luminal side only. In these experiments, the addition of CO_2 or the weak acids frequently resulted in rapid inhibition of the H^+ current.

* $P < 0.05$ compared with the control value.

increasing the transport rate is the undissociated weak acid, which would be expected to cause cytosolic acidification. The increase in H^+ secretion induced by butyrate or acetate was significantly reduced by pretreatment of the bladders with 0.1 mM colchicine (Table 1a), an agent that reduces the effect of CO_2 on exocytosis^{2,10}.

To provide direct evidence for exocytotic fusion induced by the weak acids, we measured their effect on the secretion of fluorescent dextran (fluorescein isothiocyanate (FITC) dextran) previously endocytosed by the bladder (see Fig. 1 legend for assay details). Addition of 5 mM butyrate stimulated secretion of FITC dextran simultaneously with an increase in H^+ secretion (Fig. 1b). As with CO_2 (Fig. 1a), the fusion event was transient whereas the increase in H^+ transport was constant, implying that the stimulation of H^+ transport was a result of the insertion of H^+ ATPases which continue to turn over and pump protons.

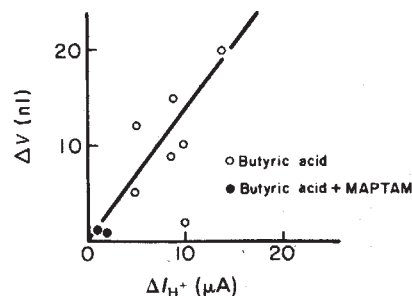


Fig. 2 Correlation of the volume of fluorescent dextran released with the rise in H^+ secretion. Known volumes of the fluorescent dextran loading solution were injected into the perfusion stream. The changes in the fluorescence signal were plotted against the volume injected to obtain a calibration curve from which the secreted volume was calculated.

There was excellent correlation between the secreted volume, a function of the number of vesicles which fused, and the increase in H^+ current, which is some function of the number of pumps in the membrane (Fig. 2). These results provide strong evidence that butyrate, like CO_2 , increases H^+ pumps in the membrane. Similar studies (not shown) showed that acetate also causes fusion of vesicles.

To measure the cell pH of individual mitochondrion-rich cells, we mounted turtle bladders on the stage of a fluorescence microscope and stained them with 6-carboxyfluorescein diacetate (6-CF). Because these cells are enriched in carbonic anhydrase, which is a potent esterase, they preferentially take up the dye and hence appear brighter than the other cell type (Fig. 3e). Measurement of the cell pH (using a microspectrofluorometric method described in Fig. 3 legend) showed that these cells are more alkaline by ~ 0.5 pH units than the principal cells, as might be expected from H^+ -secreting cells. Addition of CO_2 or butyrate acidified the cytosol with a similar time course to that of the effect of these agents on fusion and H^+ transport (compare Figs 1 and 3). That these cells can maintain concentrations of H^+ and 6-CF different from their neighbours implies that they do not have patent gap junctions, a finding that suggests a separate developmental history of the two cell types.

Fusion of vesicles is frequently mediated by changes in cell calcium and recent studies suggest that CO_2 can alter cell calcium activity¹³⁻¹⁶. However, the effect of CO_2 varies in different cell types; in some cells there is an increase¹⁴ while in others CO_2

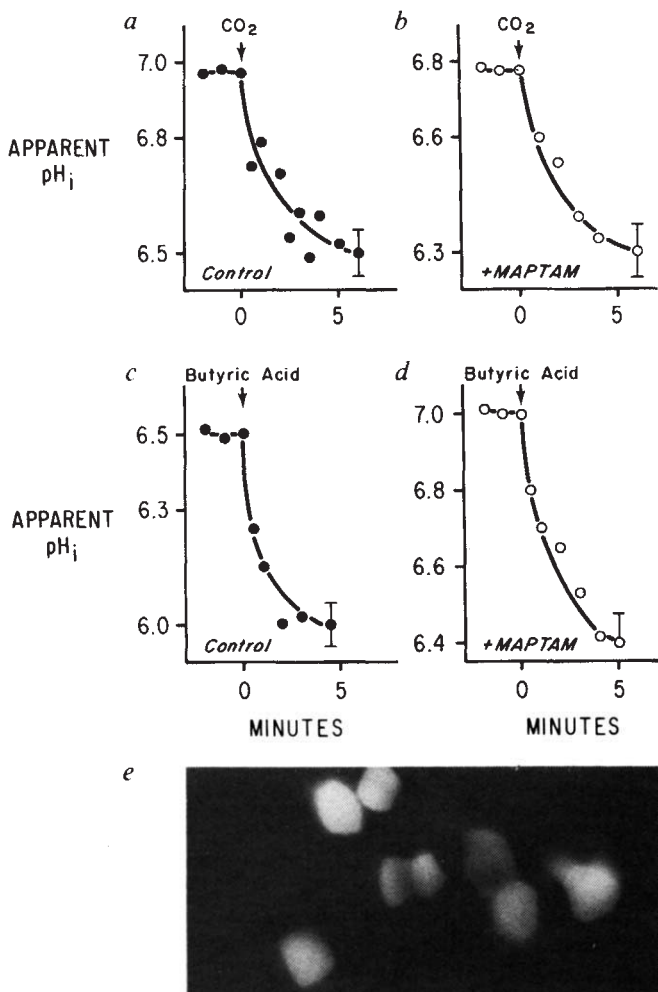


Fig. 3 *a-d*, Graphs showing the average drop in intracellular pH when the serosal perfusate was changed from CO₂-free Ringer's pH 7.5, to 5% CO₂ pH 7.5 (*a*, *b*) or 5 mM butyric acid Ringer's pH 7.5 (*c*, *d*). In *b* and *d*, the bladders were preincubated with 50 μ M MAPTAM for 1 h. *e*, Turtle bladder epithelium stained with a pH-sensitive-fluorescent dye, 6-carboxyfluorescein diacetate (6-CF). Pieces (1 cm²) of bladder were stretched across cut-off micro-centrifuge tubes. The luminal side of the bladders was stained for 4 min in 20 μ M 6-CF in CO₂-free Ringer's pH 7.5. The cylinders were placed luminal side down on a glass coverslip on a Zeiss epifluorescence microscope equipped with a mercury lamp and a $\times 40$ objective. The serosal side of the bladder was perfused continuously during microscopy. To measure the pH of the cytoplasm, we used a microfluorometer attached to the microscope (Farrand Optical)². Single cells were centred in the aperture of the fluorometer and were excited alternately with 490-nm and 458-nm light using interference filters. The fluorescence emission intensity was measured at 530 nm with the microfluorometer; aperture, 22 μ m. A calibration curve of the ratios of fluorescein intensities at the two exciting wavelengths against pH was obtained using 1 μ M 6-CF dissolved in 0.14 M KCl, 1 mM MgCl₂ from pH 5.5 to 8.0. There were 7-12 experiments for each condition. The standard deviation, although shown only for the extremes, was the same for all points. The measurement of pH in a single cell by our method is subject to several problems relating largely to light scattering and background subtraction. Because each bladder has a different thickness, it is not possible to draw conclusions about the absolute value of the pH. However, changes in an individual cell were highly reproducible.

reduces cell calcium^{13,15,16} and in squid axon the effect appears to be biphasic¹³. To measure the changes in cell calcium, we used the fluorescent calcium indicator Quin 2 in isolated suspended turtle bladder epithelial cells. Quin 2 is an EGTA analogue which fluoresces when it binds calcium^{17,18}; its ester is permeable and the active form is liberated in the cell by

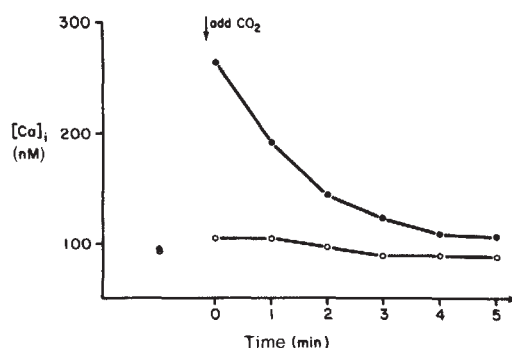


Fig. 4 Effect of CO₂ on intracellular calcium. $\bullet$, CO₂; $\circ$, no CO₂. To measure intracellular calcium, turtle bladder epithelial cells were isolated by the method of Rossier *et al.*²³, except that no collagenase digestion was used. Cells were incubated for 1 h in 40 μ M Quin 2 AM (Amersham) in CO₂-free Ringer's without calcium, pH 7.5. Cells were then washed and resuspended in CO₂-free Ringer's with 1 mM calcium. Fluorescence at excitation 340 nm with emission at 480 nm was measured with a Farrand spectrofluorometer using a 2.5-nm slit width. For each experiment, 1.5 ml of either CO₂-free Ringer's or 5% CO₂ Ringer's were added to 1.5-ml aliquots containing $\sim 1.5 \times 10^6$ cells suspended in CO₂-free Ringer's, and the cuvette was sealed. Controls and experiments were always performed on the same cell suspension. Fluorescence was measured for 5 min, at which time calibration of measured Quin 2 fluorescence to concentration of calcium was performed by the method of Tsien^{16,17} ($n = 7$, $P < 0.05$ for all points).

cytoplasmic esterases. In unstimulated cells, intracellular calcium is 95 ± 12 nM ($\pm$ s.e.). Addition of CO₂ by diluting suspended cells with CO₂-saturated buffer causes an increase in cell calcium to a peak of 264 ± 92 nM ($n = 7$, $P < 0.05$), which declines with time towards control concentrations (Fig. 4).

Although these experiments were performed in suspensions containing both cell types, we believe that the signal observed mainly results from the mitochondrion-rich cells, as they were preferentially loaded with Quin 2 through the esterase activity of carbonic anhydrase. Pretreatment of this suspension with acetazolamide, an inhibitor of carbonic anhydrase, substantially prevents the loading of these cells by Quin 2 and 6-CF. Further, acetazolamide, which increases cell pH in these cells, reduces the resting calcium level¹⁹. However, studies in individually identified mitochondrion-rich cells are needed to provide conclusive evidence for the CO₂-induced increase.

To study whether the increased cell calcium is necessary for exocytotic insertion of H⁺ pumps, we loaded the epithelium with the calcium buffer 1,2-bis-5-methyl-aminophenoxyethane-*N,N,N'*-tetraacetoxymethyl acetate (MAPTAM), another esterified but non-fluorescent EGTA derivative^{17,18}. Addition of MAPTAM decreased the H⁺ current slightly but considerably blunted the expected increase in H⁺ secretion in response to CO₂ and butyrate (Table 1*b*). However, the response was quite variable; in some bladders there was still a small increase (Fig. 1*b*, *d*), while in others there was a rapid decline in the rate of H⁺ secretion. On average (Table 1*b*), MAPTAM prevents the increase in H⁺ secretion caused by CO₂ and butyrate. This effect is due to inhibition of fusion (Fig. 1*b*, *d*). MAPTAM has no effect on the cytoplasmic acidification caused by CO₂ or butyrate (Fig. 4*b*, *d*).

These results show that CO₂ causes exocytotic insertion of H⁺ pumps initially by lowering cell pH, which in turn increases cytosolic calcium. The MAPTAM experiments suggest that the increased cell calcium is necessary for exocytosis. In preliminary experiments, we find that the cell pH, when lowered by CO₂, relaxes back to the original level in control cells but not in cells where the fusion event has been inhibited by removal of extracellular calcium. These results and the ubiquity of this H⁺ ATPase in eukaryotic cells suggest that rapid insertion may be a new mechanism of cell pH regulation in addition to the well-studied one mediated by Na/H exchange²⁰. In acid-transporting epithelia, this process would be modified only to the extent that

fusion occurs at one cell membrane. Further, recent studies on glucose-mediated insulin release²¹ and the effects of CO₂ on vasopressin-induced water permeation²² suggest that cell acidification may be a more widespread stimulus for exocytosis than previously thought.

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1. Tycko, B. & Maxfield, F. R. *Cell* **28**, 643-651 (1982).
2. Gluck, S., Cannon, C. & Al-Awqati, Q. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4327-4331 (1982).
3. Gluck, S., Kelly, S. & Al-Awqati, Q. *J. biol. Chem.* **257**, 9230-9233 (1982).
4. Forgacs, M., Cantley, L., Wiedemann, B., Altstiel, L. & Branton, D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1300-1303 (1983).
5. Stone, D. K., Xie, X.-S. & Racker, E. *J. biol. Chem.* **258**, 4059-4062 (1983).
6. Galloway, C. J., Dean, G. E., Marsh, M., Rudnick, G. & Mellman, I. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3334-3338 (1983).
7. Glickman, J., Croen, K., Kelly, S. & Al-Awqati, Q. *J. Cell Biol.* **97**, 1303-1308 (1983).
8. Rees-Jones, R. & Al-Awqati, Q. *Biochemistry* **23**, 2336-2340 (1984).
9. Gluck, S. & Al-Awqati, Q. *J. clin. Invest.* **73**, 1704-1710 (1984).
10. Schwartz, G. J. & Al-Awqati, Q. *J. clin. Invest.* (in the press).
11. Reeves, W., Gluck, S. & Al-Awqati, Q. *Kidney Int.* **23**, 232 (Abstr.) (1983).
12. Steinmetz, P. R. *Physiol. Rev.* **54**, 890-956 (1974).
13. Baker, P. F. & Honerjager, P. *Nature* **273**, 160 (1978).
14. Lea, T. J. & Ashley, C. C. *Nature* **175**, 236-238 (1978).
15. Lorenzen, M., Lee, C. O. & Windhager, E. E. *Fedn Proc.* **41**, 1350 (1982).
16. Alvarez-Leefmans, F. J., Rink, T. J. & Tsein, R. Y. *J. Physiol., Lond.* **315**, 531-548 (1981).
17. Tsein, R. Y. *Biochemistry* **19**, 2396-2403 (1980).
18. Tsein, R. Y., Pozzan, T. & Rink, T. J. *J. Cell Biol.* **94**, 325-334 (1982).
19. van Adelsberg, J. S. & Al-Awqati, Q. *Kidney Int.* **27**, 290 (abstr.) (1985).
20. Roos, A. & Boron, W. *Physiol. Rev.* **61**, 296-434 (1981).
21. Cook, D. L., Ikeuchi, M. & Fujimoto, W. Y. *Nature* **311**, 269-271 (1984).
22. Lorentzen, M., Taylor, A. & Windhager, E. E. *Am. J. Physiol.* **245**, F188-F197 (1983).
23. Rossier, M., Rossier, B. C., Pfeiffer, J. & Krachenbuhl, J. P. *J. Membrane Biol.* **48**, 141-166 (1979).

The synthesis and *in vivo* assembly of functional antibodies in yeast

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The yeast *Saccharomyces cerevisiae* can synthesize, process and secrete higher eukaryotic proteins¹⁻⁵. We have investigated the expression of immunoglobulin chains in yeast and demonstrate here (1) the synthesis, processing and secretion of light and heavy chains, (2) the glycosylation of heavy chain, (3) the intracellular localization of these foreign proteins by immunofluorescence, and (4) the detection of functional antibodies in cells co-expressing both chains. This may provide the basis of a microbial fermentation process for the production of monoclonal antibodies. The co-expression of light and heavy chains in *Escherichia coli* has been reported but functional antibodies were not assembled *in vivo*^{6,7}. Furthermore, only low-level assembly of these chains was found *in vitro*.

The immunoglobulin λ and μ complementary DNAs used here were isolated from the mouse hybridomas S43 and B1-8, respectively^{8,9}. Both hybridomas were raised against the hapten 4-hydroxy-3-nitrophenyl acetyl (NP). The λ and μ cDNAs were placed under the control of the yeast 3-phosphoglycerate kinase (PGK) promoter, on pMA91 bearing the *LEU2* selectable marker¹⁰ (Fig. 1), to form pY λ for the expression of pre- λ , and pY μ for the expression of pre- μ . For co-expression of λ and μ on different plasmids in the same cell, another selectable marker was needed. pLG89 contains two selectable markers, *URA3* and *hph* (ref. 11), therefore the λ coding sequence and PGK promoter from pY λ were excised and inserted into pLG89, to produce pLG λ .

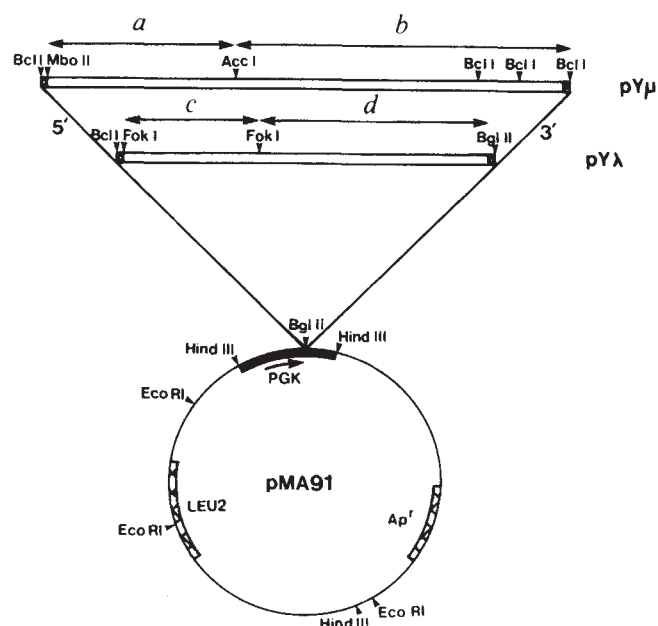


Fig. 1 Plasmids for the expression of immunoglobulin λ and μ chains in yeast. The figure shows the μ and λ inserts of pMA91 which were used to form pY μ and pY λ respectively. Solid bar, PGK sequences; the *LEU2* and *Ap^r* marker genes are cross-hatched. Sequences derived from synthetic oligodeoxyribonucleotides are indicated by horizontal bars. An arrow indicates the direction of PGK transcription.

Methods. All DNA manipulations were carried out as described elsewhere²². For construction of pY λ , the *Hind*III fragment of pAT λ 1-15 (ref. 6) bearing the λ cDNA was isolated, and cut with *Fok*I; 307-base pair (bp) *Fok*I and 512-bp *Fok*I-*Hind*III fragments were isolated (fragments *c* and *d*, respectively). Both oligodeoxyribonucleotides²²: R162 (5'-GATCAATGGCCCTG-GATT 3') and R163 (5'-GTGAAATCCAGGCCAT 3') and pCT54 (ref. 24) cut with *Bcl*I and *Hind*III, to form pCTY λ . R162/R163 recreate the 5' coding sequence, and place a *Bcl*I site immediately upstream of the initiation ATG. pCTY λ was digested with *Hind*III, blunted with *S*₁ nuclease and filled-in with T4 DNA polymerase (P-L Biochemicals); the product was ligated with *Bgl*II linkers (5'-AGAGATCTCT 3'), then digested with *Bcl*I and *Bgl*II. The fragment bearing the λ cDNA was isolated and ligated with *Bgl*II-cut pMA91, to form pY λ . The 5' λ coding sequence of pY λ was isolated and shown to be correct by nucleotide sequencing. The λ coding sequence and PGK promoter were excised from pY λ on a *Hind*III fragment, and ligated with *Hind*III-cut pLG89 to form pLG λ . For construction of pY μ , the μ cDNA was excised from pCT54 μ ²⁵ on a *Hind*III fragment, blunted (as for pCTY λ) and ligated with linker R107 (5'-TTTGTGATCAAAA 3') which contains an internal *Bcl*I site. The ligation product was digested with *Bcl*I and *Acc*I, and the fragment containing the 3' end of μ coding sequence isolated (*b*). Only the *Bcl*I site created by R107 will cut, for internal sites were *dam*-methylated. pCT54 μ was cut with *Mbo*II, ligated with R121 (5'-GATCAATGGGATGGAGCTGT 3') and R112 (5'-CAGCTCCATCCCAT 3') and digested with *Acc*I. The 276-bp *Bcl*I-*Acc*I fragment (fragment *a*) generated was isolated from a 5% polyacrylamide gel. pMA91 cut with *Bgl*II was ligated with both *a* and *b*, to form pY μ . The proximal end of the μ gene was isolated and shown to be correct by nucleotide sequencing^{26,27}. Yeast transformations were carried out as described elsewhere²⁸. pY λ and pY μ were individually transformed into MD46 (*a*/+ *pep4.3* *leu2* *arg5.6* + + *trp1* + *rad52* *ade1* + *his3* + +; Melanie Dobson, personal communication); pY μ and pLG λ were individually transformed or co-transformed into X4003-5B (*a* *leu2* *ade1* *his4* *met2* *ura3* *trp5* *gal1*; Yeast Genetic Stock Center, Berkeley, California). Cells containing pY λ or pY μ were selected for by growth in the absence of L-leucine, and pLG λ cells selected for by growth in the absence of uracil.

Cell extracts were subjected to Western blot analysis, using antisera to λ or μ proteins. MD46 cells containing pY λ were found to contain an immunoreactive protein (Fig. 2a, lane 3) that co-migrates with authentic B1-8 λ (Fig. 2a, lane 1) and with the mature λ protein synthesized by *E. coli* cells containing

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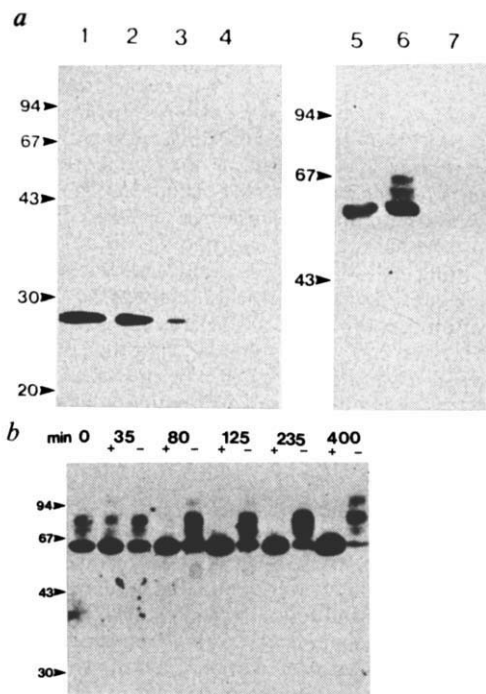


Fig. 2 Analyses of λ and μ proteins in yeast cell extracts. Cells were grown in YMM (2% (w/v) glucose, 0.67% (w/v) Difco yeast nitrogen base without amino acids) to $A_{660} = 1.0$, at 30 °C with shaking, then collected by centrifugation. Cell pellets were disrupted by vortexing with glass beads (40 mesh, BDH) in 50 mM Tris-HCl pH 7.6, 1 mM EDTA, and diluted in sample buffer to 1.5% (w/v) SDS, 2.5% (v/v) β -mercaptoethanol, 5% (v/v) glycerol. Samples were subjected to SDS-polyacrylamide gel electrophoresis²⁹ and transferred to 0.45- μ m nitrocellulose and Western-blotted^{30,31} with either rabbit anti-mouse λ antibody (Miles) or rabbit anti-mouse IgM (Bionetics), and challenged with affinity-purified iodinated protein A ($2 \mu\text{Ci ml}^{-1}$; Amersham). Migration of yeast λ and μ was compared with that of B1-8 protein and of mature λ and μ synthesized in *E. coli*⁶. **a**, Lanes 1-4, samples blotted with anti- λ antibody: lane 1, B1-8; lane 2, bacterial λ ; lane 3, pY λ /MD46 cell extract; lane 4, MD46 cell extract. Lanes 5-7, samples blotted with anti-IgM: lane 5, bacterial μ ; lane 6, pY μ /MD46 cell extract; lane 7, MD46 cell extract. Arrowheads indicate the positions of markers (Pharmacia): phosphorylase b, 94 K; bovine serum albumin, 67 K; ovalbumin, 43 K; carbonic anhydrase, 30 K; soybean trypsin inhibitor, 20.1 K. For secretion studies, cells were also grown in YPED (1% (w/v) Difco Bacto yeast extract, 2% (w/v) Difco Bacto peptone, 2% (w/v) glucose) and all cultures were buffered with potassium phosphate pH 7.0. In these conditions, we were able to detect the ADH activity in extracts from $<0.005 A_{660}$ units of cells, which would represent lysis of $<0.5\%$ of a culture at $A_{660} = 1.0$. At such concentrations, ADH activity remained stable for up to 3 h at 30 °C. Medium supernatants were assayed for ADH by mixing in a cuvette 1 ml of supernatant with 0.3 ml of 500 mM potassium phosphate pH 7.0, 100 μl 30 mM NAD (reduced form; Sigma), 0.3 ml 30 mM acetaldehyde (BDH) and 1.3 ml double-distilled water. The decrease in A_{340} was measured with a Gilford spectrophotometer at 30 °C. **b**, Demonstration of μ glycosylation in yeast. Duplicate 50-ml YMM cultures of MD46 containing pY μ were grown in 250-ml baffled flasks, with shaking at 30 °C to $A_{660} = 1.0$ then split into duplicate 20-ml cultures. To one culture, designated '+', we added tunicamycin^{12,32} to a concentration of $15 \mu\text{g ml}^{-1}$, in 30 μl of dimethyl sulfoxide (DMSO), and to the other culture, designated '-', we added 30 μl of DMSO alone. Cultures were left shaking at 30 °C, and samples taken at various times from both cultures. Cell extracts were prepared and Western-blotted with rabbit anti-mouse IgM and iodinated protein A. Alternating + and - tunicamycin samples are shown and the times of sampling given above the lanes in min. The 0-min sample was taken immediately before DMSO addition. Cells harbouring pY λ were treated similarly, and no difference was found between cultures with or without tunicamycin, when Western-blotted with anti- λ antiserum (data not shown). This result was expected as λ is a non-glycosylated protein in mammalian cells.

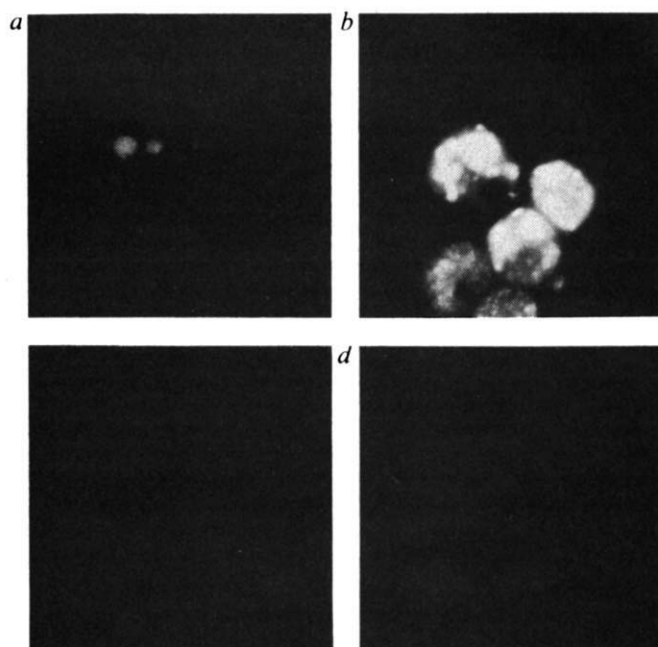


Fig. 3 Intracellular localization of λ and μ protein. The figure shows MD46 cells either untransformed (**c**, **d**), or transformed with pY μ (**a**) or pY λ (**b**); **a** and **c** were incubated with fluorescein-conjugated anti- μ ; **b** and **d** with rhodamine-conjugated anti- λ . $\times 700$.

Methods. Cells were grown in minimal media and converted to spheroplasts with zymolyase 20,000 (50 μg per A_{660} unit in 0.5 ml; Miles), in 1.2 M sorbitol, 50 mM potassium phosphate pH 7.0, 15 mM β -mercaptoethanol, 10 mM EDTA, at 30 °C for 30 min. Spheroplasts were washed in 1.2 M sorbitol, and deposited on glass microscope slides using a cytocentrifuge, then fixed in 5% (v/v) acetic acid/95% (v/v) ethanol, at -20 °C overnight. Slides were rehydrated and washed thoroughly in phosphate-buffered saline (PBS), before being stained with 0.1 mg ml^{-1} of fluorescein isothiocyanate-labelled affinity-purified goat anti-mouse IgM or tetramethylrhodamine isothiocyanate-labelled affinity-purified goat anti-mouse λ (both from Southern Biotechnology Associates, Alabama). Slides were washed in PBS, and mounted in glycerol containing 1,4-diazabicyclo(2.2.2)octane, before examination by ultraviolet microscopy.

a gene for mature λ (ref. 6) (Fig. 2a, lane 2). MD46 cells containing pY μ were found to contain three immunoreactive species (Fig. 2a, lane 6). The predominant yeast μ -immunoreactive band had a relative molecular mass (M_r) of $\sim 63,500$ (63.5 K) consistent with it being mature, non-glycosylated μ , and co-migrated with the mature μ synthesized by *E. coli* cells containing a gene for mature μ (ref. 6) (Fig. 2a, lane 5). In addition, two diffuse bands of greater relative molecular mass were found in MD46 cells containing pY μ (Fig. 2a, lane 6). The mature, glycosylated μ of B1-8 has a $M_r \sim 70$ K (not shown).

We conclude that the λ immunoreactive species is mature λ , and the ~ 63.5 K μ species is mature, non-glycosylated μ . On the basis of relative molecular mass, the signal sequences of both proteins have probably been cleaved. The higher- M_r μ bands were shown by two criteria to be glycosylated μ . First, the higher M_r μ was lost from cells treated with tunicamycin, an inhibitor of *N*-linked glycosylation¹². In cultures without tunicamycin, the amount of higher- M_r μ increased during the time course examined, up to 235-min sample, after which the culture reached stationary phase, and the level of μ decreased (Fig. 2b). In cultures containing tunicamycin, the 63.5 K μ species accumulated with this species alone being found after 80 min of tunicamycin treatment. In addition, the higher- M_r form of μ was reduced in size to that of mature, non-glycosylated μ from *E. coli*, when treated with trifluoromethane sulphonic acid, which removes *N*-linked glycosyl groups with high efficiency¹³ (data not shown). We conclude that a significant proportion of yeast μ is *N*-glycosylated. However, the yeast

and mammalian μ will probably differ in carbohydrate composition, especially if the yeast μ has outer-chain oligosaccharides^{14,15}.

Medium supernatants from cultures of MD46 cells transformed with either pY λ or pY μ were found to contain immunoreactive λ or μ protein, respectively. This was shown to be genuinely secreted material, rather than arising from cell lysis, by demonstrating the absence of a cytoplasmic enzyme, alcohol dehydrogenase (ADH), from the supernatants (see Fig. 2 legend). In yeast minimal medium (YMM), up to 10% of λ and 5% of μ in MD46 cultures at $A_{660} \sim 1.0$ was found in the medium supernatant, as detected by enzyme-linked immunosorbent assay (ELISA)²⁵. In YPED medium (Fig. 2 legend) up to 40% of λ and 15% of μ was found in this fraction. Transformed X4003-5B cells generally yielded higher levels of secreted material. The immunoreactive λ material from the medium supernatants of cultures containing pY λ was shown, by Western blotting, to co-migrate with intracellular λ (data not shown). The secreted μ has not yet been examined.

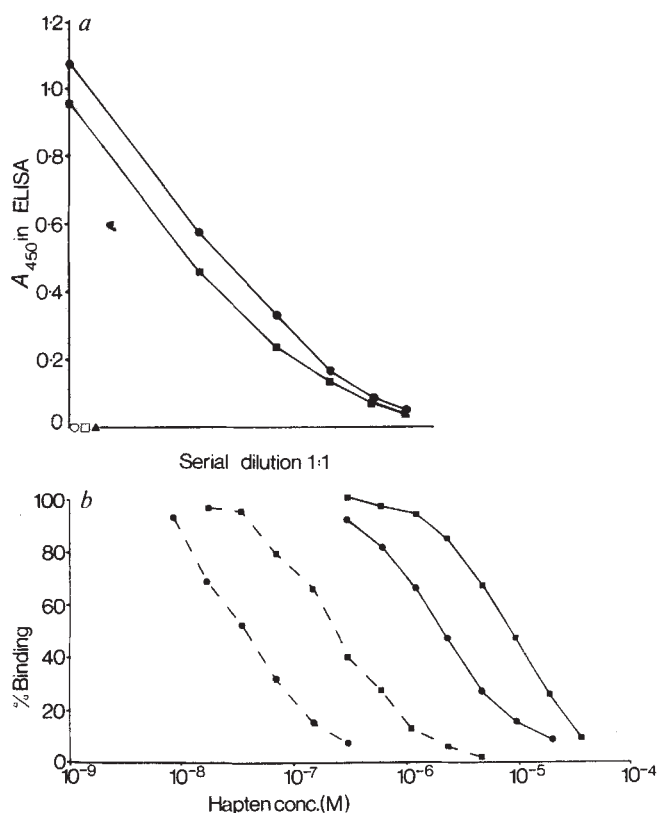


Fig. 4 Analysis of functional antibodies in yeast. Transformed X4003-5B cells were grown in 100-ml volumes of YMM, supplemented with 50 mg l⁻¹ of L-tryptophan, L-histidine and L-methionine, and 30 mg l⁻¹ of adenine sulphate in 500-ml flasks. Strains not containing pLG λ were supplemented with 30 mg l⁻¹ uracil, and those not containing pY μ with 50 mg l⁻¹ L-leucine. Cells were collected by centrifugation, then washed and resuspended in 25 mM Tris-HCl pH 8.0, 1 mM EDTA, 5% (v/v) glycerol, 0.1% (v/v) Nonidet-P40, 1 mM phenylmethylsulphonylfluoride (extraction buffer), before being lysed by glass bead disruption. 'Insoluble' material was pelleted in a microfuge for 12 min at 4 °C, and the 'soluble' supernatant fraction removed. The soluble fraction was analysed in a two-site sandwich ELISA (NIP assay) which detects μ -chain binding to haptenylated bovine serum albumin (NIP-caproate-BSA)⁶; this assay is sensitive to 60 pg of B1-8 IgM. **a**, Specific hapten binding was examined in the NIP assay of the soluble fraction of pLG λ + pY μ -transformed cells (●, ○), pY μ -transformed cells (▲) and B1-8 (■, □), in the absence (solid symbols) and presence (open symbols) of 30 μ M free NIP-caproate. **b**, The heteroclitic nature of yeast antibody activity (●) in soluble fractions from pLG λ + pY μ -transformed cells and B1-8 (■) was examined by comparing binding in the presence of free NIP-caproate (---) and NP-caproate (—).

The intracellular locations of λ and μ proteins in yeast were examined by staining fixed yeast spheroplasts with fluorescein or rhodamine-conjugated antisera. We stained both MD46 and X4003-5B strains, harbouring pY μ , pY λ , pLG λ or pYMA λ . pYMA λ is a pMA91 derivative encoding the mature λ protein, that is, Met-mature λ , not including the signal sequence. Cells containing pY μ (Fig. 3a), pYMA λ (Fig. 3b), pY λ or pLG λ showed a discrete immunofluorescence localized in bodies that appeared on the basis of their morphology to be vacuoles^{16,17}. Cells containing pYMA λ showed a much greater accumulation of stain than those containing pY λ ; this may be explained by the observation that the intracellular concentration of mature λ from pYMA λ is up to fourfold greater than that of pre- λ from pY λ , in MD46, by ELISA. Further experiments are required to identify unequivocally the sites of accumulation of immunoreactive λ and μ proteins. If these foreign proteins are being localized in the vacuole, they could be transported there by the secretory pathway^{18,19}, however, the detection of mature λ from pYMA λ in the same structures calls this into question—the cell may be directing these proteins to the vacuole for degradation²⁰.

Soluble extracts of X4003-5B cells transformed with one or both of pY μ and pLG λ were prepared and analysed by an ELISA that detects binding of B1-8 to solid-phase hapten, in the presence and absence of competing free hapten⁶ (Fig. 4a); no specific binding was detected for extracts of cells transformed with either pLG λ or pY μ . However, extracts of cells transformed with both plasmids, and expressing both light and heavy chains, showed a strong, specific signal similar to that of the hybridoma B1-8 protein. All detectable solid-phase hapten binding was lost in the presence of 30 μ M free hapten. B1-8 is a heteroclitic antibody, and shows greater affinity for the related hapten 4-hydroxy-5-iodo-3-nitrophenyl acetyl (NIP), than for the hapten NP²¹. The yeast antibody activity also showed that higher free NP than free NIP concentrations were required to inhibit binding of antibody to solid-phase NIP (Fig. 4b). In addition, the activities of the yeast antibody and B1-8 showed similar specificity ratios (ratio of concentration of NP to NIP at 50% inhibition) of 37 and 47, respectively. The amount of μ protein in the soluble extract of cells containing pLG λ and pY μ was determined by ELISA, and using B1-8 as a standard, the specific activity of the yeast antibody was found to be $\sim 0.5\%$. ELISA showed that at $A_{660} = 1.0$, there was ~ 500 ng of B1-8 λ equivalent, and 15 ng of B1-8 μ equivalent per ml. No significant amounts of NIP binding activity have been found in concentrated culture media (data not shown).

On the basis of the specific activity of the soluble-fraction yeast antibodies, the efficiency of assembly of functional antibodies is low, although the antibodies show both specific hapten binding and heterocliticity. It will be interesting to characterize further the yeast antibodies in the soluble fraction and to determine whether or not most of the immunoglobulin protein from the insoluble fraction ($\sim 75\%$ of total) is also assembled into functional antibodies.

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- Hitzeman, R. A. *et al. Science* **219**, 620-625 (1983).
- Rothstein, S. J., Lazarus, C. M., Smith, W. E., Baulcombe, D. C. & Gatenby, A. A. *Nature* **308**, 662-665 (1984).
- Valanzuela, P., Medina, A., Rutter, W. J., Ammerer, G. & Hall, B. D. *Nature* **298**, 347-350 (1982).
- Hitzeman, R. A. *et al. Nucleic Acids Res.* **11**, 2745-2763 (1983).
- Brake, A. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4642-4646 (1984).
- Boss, M. A., Kenten, J. H., Wood, C. R. & Emtage, J. S. *Nucleic Acids Res.* **12**, 3791-3806 (1984).
- Cabilly, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 3273-3277 (1984).
- Bothwell, A. L. M. *et al. Cell* **24**, 625-637 (1981).
- Bothwell, A. L. M. *et al. Nature* **298**, 380-382 (1982).
- Mellor, J. *et al. Gene* **24**, 1-14 (1983).
- Gritz, L. & Davies, J. *Gene* **25**, 179-188 (1983).

12. Mahoney, W. C. & Duskin, D. *J. biol. Chem.* **254**, 6572-6576 (1979).
13. Edge, A. S. B., Faltynek, C. R., Hof, L., Reichert, L. E. & Weber, P. *Analyt. Biochem.* **118**, 131-137 (1981).
14. Chapman, A. & Kornfeld, R. *J. biol. Chem.* **254**, 816-823 (1979).
15. Ballou, C. E. in *The Molecular Biology of the Yeast Saccharomyces, Metabolism and Gene Expression* (eds Strathern, J. et al.) 335-360 (Cold Spring Harbor Laboratory, New York, 1982).
16. Schwencke, J. *Physiol. Vég.* **15**, 491 (1977).
17. Matile, P. A. *Rev. Pl. Physiol.* **29**, 193 (1978).
18. Schekman, R. & Novick, P. in *The Molecular Biology of the Yeast Saccharomyces, Metabolism and Gene Expression* (eds Strathern, J. et al.) 361-398 (Cold Spring Harbor Laboratory, New York, 1982).
19. Stevens, T., Esmon, B. & Schekman, R. *Cell* **30**, 439-448 (1982).
20. Lin, C.-J., Chopra, A. K., Strnadova, M. & Chaloupka, J. *FEMS Microbiol. Lett.* **21**, 313-317 (1984).
21. Imanishi, T. & Makela, O. *Eur. J. Immun.* **3**, 323-330 (1973).
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
23. Patel, T. P. et al. *Nucleic Acids Res.* **10**, 5605-5620 (1982).
24. Emtage, J. S. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3671-3675 (1983).
25. Wood, C. R., Boss, M. A., Patel, T. P. & Emtage, J. S. *Nucleic Acids Res.* **12**, 3937-3950 (1984).
26. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
27. Messing, J., Crea, R. & Seeburg, P. H. *Nucleic Acids Res.* **9**, 302-321 (1981).
28. Beggs, J. *Nature* **275**, 104-109 (1978).
29. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
30. Burnette, W. N. *Analyt. Biochem.* **112**, 195-203 (1981).
31. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
32. Onishi, H. R., Kacz, J. S. & Lampen, J. O. *J. biol. Chem.* **254**, 11943-11952 (1979).

Functional modifications of cytotoxic T-lymphocyte T200 glycoprotein recognized by monoclonal antibodies

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Plasma membrane glycoproteins of cytotoxic T lymphocytes (CTLs) are involved in the binding to and subsequent destruction of appropriate target cells¹⁻³. The electrophoretic profile of surface proteins of mature CTLs, particularly those of high relative molecular mass (M_r), is markedly different from that of naive peripheral T cells or non-cytolytic T cells⁴⁻⁷, suggesting the possible involvement of these molecules in the activation of CTLs and/or in the lytic process itself. By generating monoclonal antibodies to cell-surface proteins of CTL clones, we have now detected CTL-specific modifications in one of these high- M_r membrane proteins, T200. Although forms of T200 are found on a wide variety of cell types, the neoantigenic determinants recognized by our antibodies are present exclusively on activated T cells and in high concentrations only on CTLs. Furthermore, the expression of the modifications recognized by our antibodies is influenced by soluble factors and also seems to have functional significance, as monoclonal antibodies specific for these novel epitopes block cytolytic activity.

Monoclonal antibodies with specificity for CTL surface recognition structures were produced by immunizing BALB.B mice repeatedly (intraperitoneally) with a C57BL/6-derived CTL clone, B3.3, which is specific for a BALB minor histocompatibility antigen in association with H-2K^b. Spleen cells of immune mice were fused to a myeloma partner, P3-X63 Ag8.653, and the resulting hybridoma supernatants were screened for the ability to block specific target lysis by B3.3. Figure 1a depicts the blocking activity of two of these monoclonal antibodies, CT1 and CT2, on clone B3.3 in conditions of saturating antibody. CT1 and CT2 (which are both IgM antibodies) efficiently block specific killing of BALB.B target cells by clone B3.3, CT2 being the more efficient of the two antibodies. In this regard, CT2 was as efficient as the anti-Lyt-2 monoclonal antibody, 53.6.72. Antibody I3/2.3, which is specific for the T200 molecule⁸, did not significantly affect specific lysis by this CTL clone.

To determine whether CT1 and CT2 were able to block CTL-mediated killing in conditions not requiring antigen-specific recognition on the part of the CTLs, we tested their ability to inhibit lectin-dependent cell-mediated cytotoxicity (LDCC). Both antibodies were highly efficient as inhibitors of

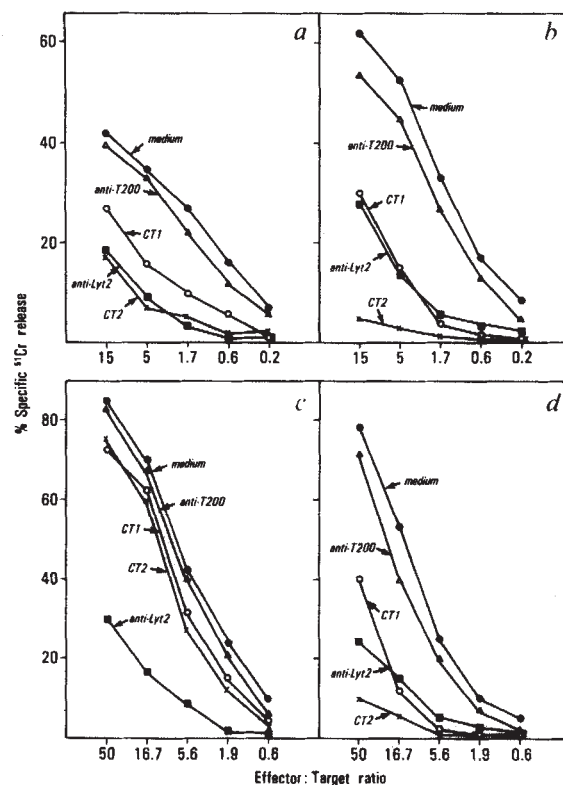


Fig. 1 Inhibition by CT antibodies of cytotoxicity mediated by cloned CTLs and MLC-derived CTLs. Serial dilutions of effector cells were incubated in 96-well round-bottomed microtitre plates for 30 min at 4 °C with saturating levels of CT1 (○), CT2 (×), I3/2.3 (△), anti-T200, kindly provided by Dr Ian Trowbridge, Salk Institute), 53.6.72 (■), anti-Lyt-2, or medium (●). Effector cells were: a, b, CTL clone B3.3 (a C57BL/6 clone derived by limiting dilution and specific for a BALB minor histocompatibility antigen in association with H-2K^b); c, C57BL/6 spleen cells stimulated for 5 days with irradiated DBA/2 spleen cells (5×10^6 cells of each per well in 24-well plates) in RPMI 1640 culture medium supplemented with 5% fetal calf serum; d, MLC cells derived as in c but restimulated (5×10^5 responders and 5×10^6 stimulators per well) every 7 days for 4 weeks in medium containing 5% rat Con A supernatant (RCS). ⁵¹Cr-labelled target cells (1×10^4) were as follows: a, 3-day BALB.B Con A blasts; b, P815 H-2^d mastocytoma cells with the addition of $10 \mu\text{g ml}^{-1}$ PHA; c, d, P815 cells. After the addition of target cells, the plates were centrifuged for 3 min at 800 r.p.m. to initiate cell contact and incubated at 37 °C for 2 h. Per cent specific lysis was calculated as $100 \times [(c.p.m. \text{ released with effectors}) - (c.p.m. \text{ released alone})] / [(c.p.m. \text{ released by detergent}) - (c.p.m. \text{ released alone})]$. Spontaneous release of P815 target cells was 5% and that of BALB.B Con A blasts 18%. No significant lysis was observed of C57BL/6 Con A blasts (a), of P815 cells without the addition of PHA (b), or of EL4 cells (c, d).

phytohaemagglutinin (PHA)-dependent killing of P815 tumour cells by clone B3.3 (Fig. 1b). CT1 was comparable with anti-Lyt-2 in blocking LDCC, while the anti-T200 antibody did not inhibit lysis (Fig. 1b). (Note that CT1 and CT2 do not bind to the target cells used in these assays; see below.) We also found that five out of five independently isolated CTL clones of various target specificities were blocked by both antibodies. Thus, it was apparent that CT1 and CT2 were not clone-specific or anti-idiotypic in their reactivity. More surprising results were obtained when we examined their effect on heterogeneous populations of CTLs generated in mixed lymphocyte culture (MLC). When the effects of these antibodies on the lytic ability of a primary MLC were tested (Fig. 1c), only minimal inhibition of specific lysis was observed using CT1, CT2 or the control antibody I3/2.3 (anti-T200), while 53.6.72 (anti-Lyt-2) significantly inhibited lysis. However, quite surprisingly, in inhibition assays using as effectors CTLs from a long-term MLC, CT1 and CT2 produced a significant decrease in specific lysis (Fig. 1d). This MLC (and others similarly blocked by CT1 and CT2) were

Table 1 CT antigen expression in cytolytic and non-cytolytic T-cell populations and in naive cell populations

Cell source	Strains represented	Reactivity
Cytotoxic T-cell clones and long-term MLC cells (15)	C57BL/6, SJL, BALB.B, BALB/c, B10.D2, B10.BR	++++
Non-cytolytic T-cell clones (11)	C57BL/6, B10.D2, CAF ₁ , B10.A	+/-
Non-cytolytic T-cell hybrids (10)	B10.A, B10.D2	-, +/-
Primary MLC cells	C57BL/6, BALB.B	++
Thymocytes	C57BL/6, BALB.B	-
Lymph node T cells	C57BL/6, BALB.B	-
Bone marrow	C57BL/6	-
Spleen	C57BL/6	-

Reactivity of CT1 and CT2 was determined by fluorescence flow cytometry or fluorescence microscopy. CTL clones, long-term MLC cells and non-cytolytic T-cell clones were propagated by restimulation with the appropriate irradiated spleen cells in medium containing 5% supernatant derived from Con A-stimulated rat spleen cells (RCS) and 50 mM α -methyl mannoside. Non-cytolytic T-cell hybrids and primary MLC cells were grown in medium without RCS. MLCs were established by co-culturing 5×10^6 responder spleen cells per 2 ml culture with 5×10^6 irradiated (2,000 rad, ^{137}Cs source) stimulator spleen cells. Primary MLC cells were tested for CT reactivity 5 days after stimulation and long-term MLC cells were tested 4 days after the fifth *in vitro* restimulation. Viable cells were isolated by passage over Ficoll-Isopaque, resuspended in phosphate-buffered saline with 0.1% NaN₃ and 0.1% bovine serum albumin at a concentration of $1 \times 10^7 \text{ ml}^{-1}$. Cells (1×10^6) were incubated with 100 μl of a 1:100 dilution of ascites fluid of antibodies CT1 or CT2 at 4°C for 30 min. After washing, the cells were incubated for 30 min at 4°C with a fluorescein-conjugated affinity-purified F(ab')₂ rabbit anti-mouse reagent (Cappel Laboratories). After washing, the cells were resuspended at $1 \times 10^6 \text{ cells ml}^{-1}$ for cytofluorimetric analysis or were placed directly on slides for fluorescence microscopic analysis. Numbers in parentheses indicate the number of individual clones tested. For comparison of relative fluorescence intensities, see Fig. 3.

cultured in the presence of supernatants containing interleukin-2 (IL-2), as were our CTL clones. Only weak blocking of long-term MLC cells was observed without the addition of exogenous IL-2 to the cultures. This dichotomy seems to result from differential expression of CT antigens on MLC cells cultured with or without lymphokine-rich supernatants (see below).

As these antibodies clearly were reacting with CTLs, we also investigated the presence of the antigens recognized on other cell populations. By screening a large panel of T-cell clones for reactivity with CT1 and CT2, we determined that only T cells of CTL lineage expressed significant levels of the antigen(s) recognized by the CT monoclonal antibodies (Table 1). Seventeen independent CTL clones expressed high levels of CT antigen whereas 11 non-cytolytic T-cell clones and 10 non-cytolytic T-cell hybrids either did not express the CT determinants or expressed them only weakly. Importantly, both CTL clones and non-cytolytic T-cell clones were cultured identically in the presence of antigen and IL-2-containing supernatants. Furthermore, the T-cell clones tested represent a wide variety of specificities and strains of origin, indicating that the genetic background of the T cells probably does not influence CT antigen expression. In fact, these antibodies are autoantibodies, as they were produced in BALB.B mice and react with BALB.B-derived CTL. Primary MLC cells express intermediate levels of CT antigen whereas thymocytes, lymph node T cells, spleen cells and bone marrow cells were unreactive with the CT antibodies. Several T-cell lymphoma lines tested also did not express the CT antigens⁹.

The characteristics of CT1 and CT2 suggested the presence of a CTL-specific differentiation or activation antigen(s) involved in CTL-induced lysis. The biochemical characteristics of the antigen recognized by these antibodies were explored by radioiodination of the CTL clone B3.3 followed by immune precipitation (Fig. 2). Precipitation with CT1 followed by SDS-

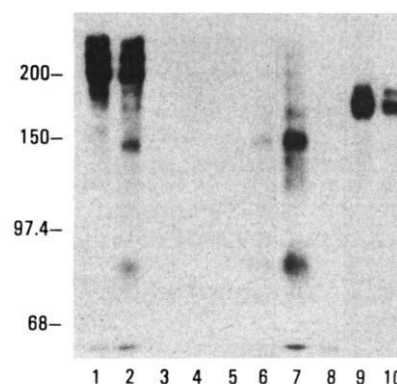


Fig. 2 Radioimmuno-precipitation by anti-T200 and CT1 antibodies. CTL clone B3.3, EL4 thymoma cells or the non-cytolytic M1s-reactive T-cell clone BB5 (kindly provided by Dr Andrew Glasebrook, Salk Institute) were labelled using Na¹²⁵I (1.0 mCi per 1×10^7 cells) by the lactoperoxidase method²⁸. After washing, the cells were resuspended in lysis buffer (phosphate-buffered saline, 1% Nonidet P-40 (NP40), 2 mM phenylmethylsulphonyl fluoride, 20 IU ml⁻¹ aprotinin, 10 mg ml⁻¹ bovine serum albumin) and incubated on ice for 30 min. The lysates were centrifuged at 13,000 g for 5 min and aliquots of the supernatant were incubated with ascites fluid containing CT1 or, in the case of I3/2.3, with the monoclonal antibody directly coupled to Sepharose 4B (a gift of Dr Ian Trowbridge). For precipitations using CT1, protein A-Sepharose 4B coated with rabbit anti-mouse immunoglobulin was used as a second-step reagent. After incubation at 4°C, the immune complexes were washed extensively with buffer (20 mM Na-phosphate, 0.5 M NaCl, 0.5% deoxycholic acid, 0.5% NP40, 0.1% SDS, 2 mM EDTA) and subsequently analysed by SDS-polyacrylamide gel electrophoresis according to the procedure of Laemmli²⁹ but using an acrylamide stock a 30% w/v acrylamide, 0.17% bis-acrylamide solution to form 7.5% acrylamide gels. The gels were dried and subjected to autoradiography. Lanes 1-8, CTL clone B3.3. Lane 1, I3/2.3 (anti-T200); lane 2, CT1; lane 3, CT1 after preclearance with CT1; lane 4, I3/2.3 after preclearance with CT1; lane 5, I3/2.3 after preclearance with I3/2.3; lane 6, CT1 after preclearance with I3/2.3; lanes 7, 8, prolonged autoradiographic exposure of lanes 6, 5, respectively. Lanes 9, 10, I3/2.3 precipitates of labelled BB5 and EL4 cell lysates, respectively. M, markers are shown $\times 10^{-3}$.

polyacrylamide gel electrophoresis (PAGE) yielded a series of proteins of high M_r as well as two proteins of relatively lower M_r (Fig. 2, lane 2). The high M_r proteins recognized by CT1 appeared similar to the molecules precipitated from the same lysates by the anti-T200 antibody I3/2.3 (Fig. 2, lane 1). CT1 precipitated proteins with apparent M_r s of 24,000 (24K), 220K and 200K and two proteins with lower apparent M_r s of 140K and 85K. Minor proteins of 190K and 180K were also occasionally observed. Precipitation with anti-T200 antibody followed by PAGE showed nearly the same high- M_r protein profile as that precipitated by CT1, with a greater amount of the 190K protein being precipitated by anti-T200. Interestingly, the 140K and 85K proteins precipitated by CT1 were never observed in anti-T200 immunoprecipitates. Although CT2 was very inefficient in immunoprecipitation experiments, Western blot analysis with both antibodies showed similar binding profiles (data not shown).

We next investigated whether the determinants recognized by CT1 and the anti-T200 antibody were present on the same molecules. Sequential immunoprecipitations were carried out using CT1 or anti-T200 to preclear lysates from surface-labelled cloned CTLs. Preclearing of the lysate from clone B3.3 with CT1 resulted in the complete removal of the antigens recognized by CT1 (Fig. 2, lane 3) and by anti-T200 (lane 4). Conversely, preclearing with anti-T200 followed by precipitation with anti-T200 or CT1 revealed that most of the high- M_r material reactive with CT1 or anti-T200 had been removed (Fig. 2, lanes 5, 6). However, the two lower- M_r proteins recognized by CT1 were not removed by preclearance with anti-T200 (Fig. 2, lanes 6, 7).

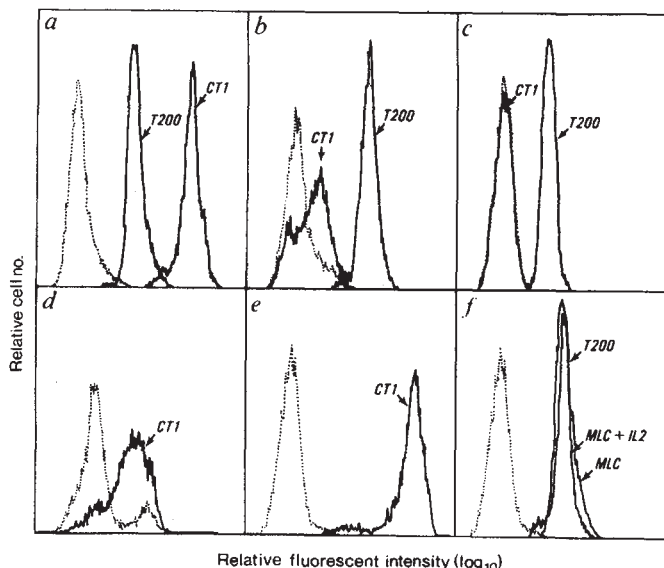


Fig. 3 The preferential reactivity of CT monoclonal antibodies with CTLs is not due to variations in cell-surface T200 concentration. *a*, CTL clone B3.3; *b*, T-helper clone D10.2; *c*, thymocytes; *d*, primary MLC; *e*, long-term MLC cultured with RCS; *f*, long-term MLC with or without the addition of RCS. Viable cells were stained as in Table 1 with CT1 or I3/2.3 (anti-T200) followed by reaction with an F(ab')₂ of a fluorescein-rabbit anti-mouse immunoglobulin (CT antibodies) or fluorescein rabbit anti-rat immunoglobulin (I3/2.3). Relative fluorescent intensities of individual cells were measured using the FACS-IV (Becton-Dickinson). Forward angle light scatter was used to exclude dead and aggregated cells. Dotted lines indicate the fluorescent profile of control antibody. Non-cytolytic T-cell clone D10.2 was provided by Dr Susan Webb.

For comparison of the T200 molecule present on CTLs with the T200 protein of other T-cell populations, the non-cytolytic T-cell clone BB5 and the EL4 thymoma were surface-labelled and the lysates immunoprecipitated with anti-T200 (Fig. 2, lanes 9, 10). PAGE analysis showed that two 175K and 190K proteins were precipitated from the surface of EL4 cells (a minor protein of 200K was also sometimes visible) and a heterogeneous protein(s) of 175–195K was precipitated from BB5. The T200 protein profile of EL4 was virtually identical with that of thymocytes and lymph node T cells. Immunoprecipitation with CT1 revealed that a minor amount of T200 (<1% of total precipitable T200) was precipitated from BB5 cell lysates while no specific precipitation of proteins by CT1 from EL4 lysates was observed⁹.

Because the CT determinants are expressed on T200 molecules, their differential expression could result from disparate T200 levels on different cell populations. To test this possibility, we analysed T-cell populations by fluorescence flow cytometry after reactions with CT or anti-T200 antibodies followed by reaction with a fluorescein-labelled secondary reagent (Fig. 3). CTL clone B3.3 expressed high levels of CT determinants (Fig. 3*a*), whereas an M1s-reactive non-cytolytic T-cell clone D10.2 (which produces IL-2 in response to antigen) exhibited very low reactivity with the CT antibody, ~25% of the cells being weakly CT⁺ (Fig. 3*b*). However, the level of cell-surface T200 on these clones was nearly identical. Thymocytes were unreactive with CT antibodies but displayed a level of T200 similar to that of the T-cell clones (Fig. 3*c*). Primary MLC cells (which were ~85% Thy 1⁺ and ~65% Lyt-2⁺) expressed intermediate levels of CT antigen, with ~60% of the cells being CT⁺ (Fig. 3*d*). In contrast, virtually all long-term MLC cells cultured with exogenous IL-2 (>95% Thy 1⁺, T200⁺ and Lyt-2⁺) expressed very high levels of CT antigen (Fig. 3*e*). The T200 concentration on long-term MLC cells cultured with or without exogenous IL-2 remained constant (Fig. 3*f*), indicating that different levels of CT antigen expression were not the result of variations in T200 expression. The shift from low to high CT antigen expression is accompanied by marked sequential biochemical changes in the T200 molecule⁹.

The T200 glycoprotein, which carries the Ly5 determinants^{10,11}, has been implicated in several immunological mechanisms^{12–14} and is a major cell-surface receptor for concanavalin A (Con A)^{15,16}. The inhibitory activity of anti-Ly5 sera or anti-T200 monoclonal antibodies on CTLs has remained a controversial topic^{17–21}. (Note that the CT antibodies bind to long-term MLC cells and T-cell clones from both B6 (Ly 5.1) and SJL (Ly 5.2) mice, Table 1.) Our results indicate that the CT antibodies are able to inhibit CTLs expressing high levels of CT antigen but are unable to block CTLs exhibiting relatively low CT antigen levels (long-term MLC cells compared with primary MLC cells; Fig. 3*d*, *e*). However, primary MLC cells do express significant levels of CT antigen whereas naive peripheral T cells and clones of T-helper cell phenotype do not. The inability of CT antibodies to inhibit primary MLC CTLs does not exclude a role for CT determinants in the general mechanism of CTL-induced lysis, as the inhibition assay may be influenced by various parameters, including antigen expression (as shown), the relative affinities of monoclonal antibodies and CTLs, and CTL recycling.

Few antigens have been described that are specific for activated T cells, and no monoclonal antibodies specific for activation determinants of a T-cell subclass have been reported until now. By selectively labelling T-cell surface glycoproteins, Kimura and Wigzell²² demonstrated the presence of a 145K protein expressed primarily on Lyt 1⁺2⁺ T cells, but drew no conclusions as to the functional role of this molecule. Whether this protein is related to the 140K protein precipitated by CT1 is unknown. The monoclonal antibody HNK-1, which is reactive with carbohydrate, defines a differentiation antigen selectively expressed on human natural killer cells^{23,24} and is therefore similar to the CT antibodies in defining a functional subset of lymphocytes. Indeed, our preliminary results suggest the CT antibodies may also be specific for cell-type-specific carbohydrate determinants. Interestingly, a variant of T200, termed T200A, selectively appears on most T cells after activation²⁵. However, unlike the antigens described here, the T200A determinants are present on molecules distinct from the T200 glycoprotein and monoclonal antibodies specific for T200A do not inhibit CTL-mediated cytotoxicity²⁵. Recent work has also indicated the presence on human T cells of high-*M_r* activation antigens distinct from the human T200 molecule^{26,27}. Thus, activation of T cells results in the appearance of proteins or antigenic determinants not present on resting T cells which are apparently involved in their subsequent functions. We propose that the maturational modifications of the T200 glycoprotein detected by our antibodies are functionally related to the lytic capability of CTLs. These alterations may act to stabilize further the interaction of CTL and target cells or may represent modifications necessary for the triggering of the lytic mechanism in fully differentiated CTLs.

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- Martz, E., Heagy, W. & Gromkowski, S. H. *Immun. Rev.* **72**, 73–96 (1983).
- Meur, S. C. et al. *J. exp. Med.* **157**, 705–719 (1983).
- Sarmiento, M. et al. *Immun. Rev.* **68**, 135–169 (1982).
- Dunlap, B. et al. *J. Immun.* **125**, 1829–1831 (1980).
- Sarmiento, M., Glasebrook, A. L. & Fitch, F. W. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1111–1115 (1980).
- Dunlap, B. et al. *Trans. Proc.* **13**, 1143–1146 (1981).
- Tung, J. S., Scheid, M. P. & Palladino, M. A. *Immunogenetics* **17**, 649–654 (1983).
- Trowbridge, I. S. *J. exp. Med.* **148**, 313–323 (1978).
- Lefrançois, L. & Bevan, M. J. *J. Immun.* (in the press).
- Omary, M. B., Trowbridge, I. S. & Scheid, M. P. *J. exp. Med.* **151**, 1311–1316 (1980).
- Siadak, A. W. & Nowinski, R. C. *J. Immun.* **125**, 1400–1401 (1980).
- Yakura, H., Shen, F. W., Bourcet, E. & Boyse, E. A. *J. exp. Med.* **157**, 1077–1088 (1983).
- Harp, J. A. & Ewald, S. J. *Cell Immun.* **81**, 71–80 (1983).
- Ralph, S. J. & Berridge, M. V. *J. Immun.* **132**, 2510–2514 (1984).
- Sitkovsky, M., Pasternack, M. S., Lugo, J. P., Klein, J. R. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1519–1523 (1984).
- Favero, J. J. et al. *Cell. Immun.* **68**, 38–52 (1982).
- Nakayama, E., Shiku, H., Stoucent, E., Oetting, H. F. & Old, L. J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1977–1981 (1979).

18. Harp, J. A., Davis, B. S. & Ewald, S. J. *J. Immun.* 133, 10-15 (1984).
19. Minato, N., Reid, L., Cantor, H., Lengyel, P. & Bloom, B. R. *J. exp. Med.* 152, 124-137 (1980).
20. Brooks, C. G., Kuribayashi, K., Sole, G. E. & Henney, C. S. *J. Immun.* 128, 2326-2335 (1982).
21. Davignon, D., Martz, E., Reynolds, T., Kurzinger, K. & Springer, T. A. *Proc. natn. Acad. Sci. U.S.A.* 78, 4535-4539 (1981).
22. Kimura, A. K. & Wigzell, H. *J. exp. Med.* 147, 1418-1434 (1978).
23. Abo, T. & Balch, C. M. *J. Immun.* 127, 1024-1029 (1981).
24. Kruse, J. *et al. Nature* 311, 153-155 (1984).
25. Sarmiento, M., Loken, M. R., Trowbridge, I. S., Coffman, R. L. & Fitch, F. W. *J. Immun.* 128, 1676-1684 (1982).
26. Hemler, M. E., Ware, C. F. & Strominger, J. L. *J. Immun.* 131, 334-340 (1983).
27. Hemler, M. E. *et al. J. Immun.* 132, 3011-3018 (1984).
28. Hubbard, A. L. & Cohn, Z. A. *J. Cell Biol.* 55, 390-405 (1972).
29. Laemmli, U. K. *Nature* 227, 680-685 (1970).

Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences

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The specificity of monoclonal antibodies provides a powerful diagnostic and therapeutic tool in investigating human neoplasia. Radiological scanning and immunotherapy with mouse tumour-specific monoclonal antibodies have been applied to patients with some success¹⁻³, but a major problem is the neutralization of the mouse antibody induced by repeated administration of heterologous antibodies. To avoid or reduce such immune reactions, chimaeric immunoglobulins consisting of mouse variable (V) and human constant (C) regions can be synthesized. We have constructed a recombinant retrovirus DNA carrying genomic heavy-chain (H) variable-diversity joining (V_H -D- J_H) and $C_{\gamma 1}$ genes from different species and show here that the chimaeric intervening sequences are spliced out precisely. This procedure provides a useful method to construct the chimaeric mouse-human immunoglobulin gene to be expressed in *Escherichia coli*, yeast and animal cells. Unexpectedly, a hidden splice donor site in the 5'-flanking region of a human V_H gene is used in place of the donor site of the leader sequence exon, resulting in the formation of the V region without the leader sequence.

We have chosen two pairs of immunoglobulin V_H and C_H genes from different species as models for the chimaeric recombinant immunoglobulin heavy-chain gene. One pair consists of the mouse $C_{\gamma 1}$ gene⁴ and a human V_H gene encoding a V_H region against the surface antigen of hepatitis B virus (HBV). These two genes were inserted into a retrovirus vector plasmid, pTK-2Δter (R)⁵ and the recombinant virus plasmid abbreviated as pSNV₁·hV_{HBV}-mC_{γ1} (Fig. 1a). The other pair, consisting of the human $C_{\gamma 1}$ gene⁶ and the mouse V_H gene of TEPC15 myeloma⁷, was inserted into pSNVΔTK (ref. 8) and the recombinant virus plasmid abbreviated as pSNV₂·mV_{T15}-hC_{γ1} (Fig. 1b). These two vectors are derived from spleen necrosis virus (SNV). As the removal of the poly(A) addition signal AATAAA of an inserted foreign gene increases the recovery of the SNV vector⁵, we removed the sequence 3' to the *HinfI* site located 39 base pairs (bp) downstream of the mouse $C_{\gamma 1}$ coding sequence and the sequence 3' to the *HhaI* site located 88 bp downstream of the human $C_{\gamma 1}$ coding sequence.

To recover infectious viruses carrying the immunoglobulin gene, pSNV₁·hV_{HBV}-mC_{γ1} and pSNV₂·mV_{T15}-hC_{γ1} DNAs were used to transfect chicken embryo fibroblasts with or without 1/10th as much DNA of a provirus clone of a non-defective helper virus, reticuloendotheliosis virus strain A (REV-A)⁹. Before transfection, pSNV₁·hV_{HBV}-mC_{γ1} DNA was digested with *SalI* to remove the pBR322 sequence. Virus production was assayed by reverse transcriptase activity¹⁰. The enzyme activity of the culture supernatants of the cells transfected with recombinant and helper viral DNAs rose to ~10-fold higher

than that of the background on day 5-7 after transfection. We next used viruses in culture supernatants recovered 7-10 days after transfection to infect fresh chicken embryo fibroblasts. Three days later, non-integrated linear viral DNA was isolated and examined for processing of the inserted DNA by Southern blot hybridization.

The parental pSNV₁·hV_{HBV}-mC_{γ1} DNA contains the 3.6-kilobase (kb) *BglII* fragment that hybridizes to both human V_{HBV} and mouse $C_{\gamma 1}$ probes (probes A and B in Fig. 1a, respectively). On the other hand, *BglII* digestion of the processed pSNV₁·hV_{HBV}-mC_{γ1} DNA should yield a 2.6-kb fragment hybridizing to both probes (Fig. 1). As shown in Fig. 2a, viral DNA from chicken embryonic fibroblasts transfected with pSNV₁·hV_{HBV}-mC_{γ1} and helper virus DNA contained the 3.6- and 2.1-kb *BglII* fragments hybridizing to both human V_{HBV} and mouse $C_{\gamma 1}$ probes, suggesting that the viral DNA contains the parental and processed forms. No significant viral DNA was recovered from transfectants without the helper virus DNA (Fig. 2a, lanes 2, 3, 7, 8).

The parental pSNV₂·mV_{T15}-hC_{γ1} DNA contains the 1.3-kb *BamHI* fragment hybridizing to the mouse V_{T15} probe (probe C) and a 3.6-kb *BamHI* fragment hybridizing to the human $C_{\gamma 1}$ probe (probe D). *BamHI* digestion of the processed pSNV₂·mV_{T15}-hC_{γ1} should yield a 1.7-kb fragment hybridizing both to probes C and D. Viral DNA isolated from transfectants by pSNV₂·mV_{T15}-hC_{γ1} and helper viruses contained the 1.7- and 1.3-kb *BamHI* fragments hybridizing to probe C (Fig. 2b, lanes 1-5) and the 1.7- and 3.6-kb *BamHI* fragments hybridizing to probe D (Fig. 2b, lanes 6-10). There are three other potential donor splice sites (J_{H2} , J_{H3} and J_{H4}) paired with the acceptor splice site of the C_{H1} exon. If the donor sites of the J_{H2} , J_{H3} and J_{H4} segments were used, we should see the 2.2-, 1.5- and 2.0-kb *BamHI* bands, respectively, hybridizing to the human $C_{\gamma 1}$ probe. There were no additional faint bands between 3.6- and 1.7-kb *BamHI* bands, excluding these possibilities and suggesting that the chimaeric immunoglobulin genes are processed at the expected intron/exon splice sites.

To analyse the structure of the progeny viruses in more detail, the inserts of the progeny viruses were cloned into Charon 28H vector, a derivative of Charon 28 (see Fig. 3 legend). The progeny viral DNAs of pSNV₁·hV_{HBV}-mC_{γ1} and pSNV₂·mV_{T15}-hC_{γ1} were digested with *BglII* and *BamHI*, respectively, and fractionated by agarose gel electrophoresis. DNA fractions containing the 2.1-kb *BglII* and 1.7-kb *BamHI* fragments were ligated into the *BamHI* site of phage Charon 28H DNA. Recombinant phages were screened by appropriate immunoglobulin gene probes (Fig. 3). Restriction enzyme cleavage analysis indicated that the eight hV_{HBV}-mC_{γ1} clones and the two mV_{T15}-hC_{γ1} clones obtained are identical with respect to restriction-site mapping.

The restriction map of the cloned hV_{HBV}-mC_{γ1} DNA is consistent with that of the processed chimaeric gene of the human V_{HBV} and mouse $C_{\gamma 1}$ gene except that the left-most *TaqI*/*XbaI* is shortened by 500 bp (Fig. 1a). Disappearance of the *BamHI* site in the 5'-flanking region of the processed hV_{HBV}-mC_{γ1} agrees with the reduction of the *TaqI*/*XbaI* fragment size, suggesting that segmental deletion occurs in the 5'-flanking region of the V_{HBV} gene. The total length of the cloned hV_{HBV}-mC_{γ1} insert is 2.1 kb, 500 bp shorter than predicted, in agreement with the size of the *BglII* fragment (2.1 kb) observed by Southern blot hybridization (Fig. 2a). The 500-bp deletion in *TaqI*/*XbaI* fragment, therefore, does not result from a cloning artefact but from genetic events during virus replication. Restriction-site mapping of the progeny mV_{T15}-hC_{γ1} clones is consistent with the loss of all introns from the parental mV_{T15}-hC_{γ1} insert.

Nucleotide sequences surrounding the junctions of the V and C gene sequences of the progeny clones are aligned with those around the splice sites of the parental V and C gene exons as shown in Fig. 3. It is clear that the V-C junctions of pSNV₁·hV_{HBV}-mC_{γ1} and pSNV₂·mV_{T15}-hC_{γ1} are processed precisely according to the GT-AG rule¹¹, indicating that interspecies pairs of exons easily allow precise removal of introns.

Nucleotide sequences of other exon junctions also demonstrate that all the introns are spliced out exactly as expected (data not shown).

We also read the sequence of the 5'-flanking region of the genomic V_{HBV} gene and find that there is a potential donor

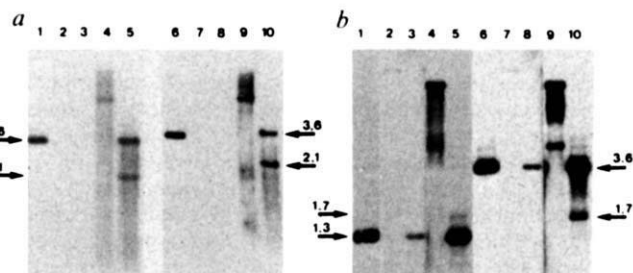
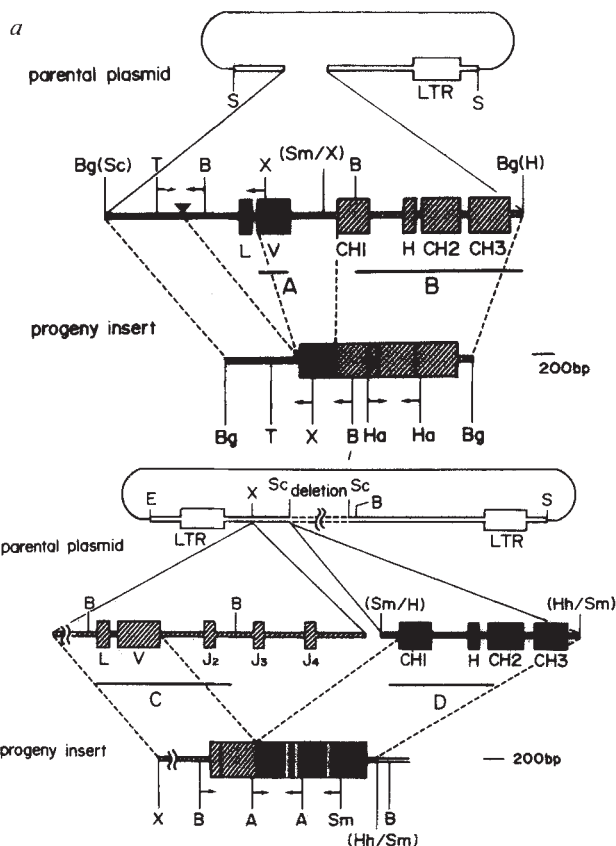
splice site, CCGGTGAGA, at 268–276 bp downstream from the *TaqI* site. Comparison of the nucleotide sequences of the parental and progeny V_{HBV} genes and their upstream regions reveals that the region extending from this potential donor site to the acceptor site of the V_{HBV} exon is removed (Fig. 3). Therefore,

Fig. 1 Structure of parental and progeny recombinant immunoglobulin genes; *a*, pSNV₁·hV_{HBV}-mC_γ1; *b*, pSNV₂·mV_{T15}-hC_γ1. Parental recombinant plasmids containing retroviral and immunoglobulin sequences are shown at the top and immunoglobulin gene sequences in progeny retroviruses at the bottom. Retrovirus, mouse and human immunoglobulin sequences are indicated by open, oblique and solid rectangles, respectively. The long terminal repeat (LTR) and exons are shown by wider rectangles and the pBR322 sequence by a solid line. Corresponding DNA segments in parental and progeny viruses are aligned by dotted lines. Solid bars indicate probes used. Directions and ranges of sequences read are shown by horizontal arrows. L, leader exon; V, V-D-J exon; H, hinge exon; C_{H1}, C_{H2} and C_{H3}, exons encoding each domain. Restriction sites are indicated as follows: A, *Ava*II; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hinf*I; Ha, *Hae*III; Hh, *Hha*I; S, *Sall*; Sc, *Sac*II; Sm, *Sma*I; T, *Taq*I; X, *Xba*I. In *a*, fragments A and B indicate the *Sau*3A/*Hha*I fragment of the human V_{HBV} gene and the *Bam*HI/*Bgl*II fragment of the mouse C_γ1 gene, respectively. The closed triangle indicates the hidden donor splice site in the *Taq*I/*Bam*HI fragment. In *b*, fragments C and D indicate the *Bam*HI fragment of the mouse V_{T15} gene and the *Sac*II fragment of the human C_γ1 gene, respectively.

Methods. Construction of pSNV₁·hV_{HBV}-mC_γ1. We cloned the V_H gene encoding the V region against the surface antigen of HBV from the Epstein-Barr-virus-transformed cell and determined the nucleotide sequence of the coding region (T. Noma and T. Honjo, unpublished). The 2.2-kb (*Sac*II/*Sma*I) fragment of the human V_{HBV} gene was prepared. The *Sac*II fragment (2.5 kb) including the mouse C_γ1 gene sequence was isolated electrophoretically from λgtWES·IgH-2 (ref. 4) and digested separately with *Xba*I and *Sma*I, and with *Sma*I and *Hinf*I. *Hinf*I digests the C_γ1 gene 30 bp upstream from the poly(A) addition signal AATAAA. We inserted the 2.2-kb *Sac*II/*Sma*I fragment of the human V_{HBV} gene and the 0.88-kb *Xba*I/*Sma*I and 0.64-kb *Sma*I/*Hinf*I fragments of the mouse C_γ1 gene into the *Bgl*II site of pTK-2ΔΔTer (R) (ref. 5). The *Xba*I site of the mouse C_γ1 gene was treated with the Klenow fragment of DNA polymerase I to make the blunt end and ligated with the *Sma*I site of the human V_{HBV} gene. The *Sac*II site of human V_{HBV} and the *Hinf*I site of mouse C_γ1 gene were filled with Klenow fragment and ligated with *Bgl*II linker (Takara Co. Ltd). The intron between the V and C exons consists of 460 bp of 3'-noncoding sequence of the V_{HBV} -D-J_H gene and 50 bp of the 5'-noncoding sequence of the mouse C_γ1 gene. Construction of pSNV₂·mV_{T15}-hC_γ1. pSNV-TK (ref. 8) was digested with *Sac*II to remove the 1.6-kb *Sac*II fragment in the *tk* gene and then recircularized with T4 ligase to give plasmid pSNVΔTK. The *Hind*III fragment of Ig_γ1-10-containing human C_γ1 gene⁶ was first subcloned into pBR322 and the plasmid then digested separately with *Hind*III and *Pst*I, and with *Pst*I and *Hha*I. (*Hha*I cleaves the C_γ1 gene 15 bp upstream from AATAAA sequence.) Equimolar amounts of the *Hind*III/*Pst*I fragment containing the C_{H1} exon and the *Pst*I/*Hha*I fragment containing the C_{H2}, hinge and C_{H3} exons were ligated together. The 1.9-kb fragment corresponding to the length of the intact *Hind*III/*Hha*I fragment of the human C_γ1 gene was purified and the ends of the fragment were filled by the Klenow fragment of DNA polymerase I. The blunt-ended DNA fragment was inserted into the *Sma*I site of pSNVΔTK and the *Xba*I-fragment containing the V_{T15} sequence⁷ then inserted into the *Xba*I site of pSNVΔTK·C_γ1. The intron between the V_{T15} and C_γ1 exons consists of the 3'-noncoding region of the V_{T15} (2 kb) containing the J_{H2}, J_{H3} and J_{H4} exons, the 5'-noncoding region of the human C_γ1 gene (200 bp) and the *Xba*I/*Sma*I fragment of pSNV-TK (200 bp).

Fig. 2 Structural analyses of nonintegrated progeny viral DNA from chicken fibroblasts infected with recombinant viruses. Non-integrated viral DNA was analysed by Southern blot hybridization using the nick-translated probes indicated. *a*, Progeny virus of pSNV₁·hV_{HBV}-mC_γ1. DNA is as follows: lanes 1, 6, parental pSNV₁·hV_{HBV}-mC_γ1; lanes 2, 3, 7, 8, progeny of pSNV₁·hV_{HBV}-mC_γ1 recovered from chicken fibroblast cells transfected without helper virus DNA; lanes 4, 5, 9, 10, progeny of pSNV₁·hV_{HBV}-mC_γ1 recovered from fibroblasts transfected with helper virus DNA. DNAs in lanes 1, 3, 5, 6, 8 and 10 were digested with *Bgl*II. Hybridization probes used: lanes 1–5, probe A; lanes 6–10, probe B described in Fig. 1. *b*, Progeny virus of pSNV₂·mV_{T15}-hC_γ1. DNA lanes 1, 6, parental pSNV₂·mV_{T15}-hC_γ1; lanes 2, 3, 7, 8, progeny of pSNV₂·mV_{T15}-hC_γ1 recovered from fibroblasts transfected without helper virus DNA; lanes 4, 5, 9, 10, progeny of pSNV₂·mV_{T15}-hC_γ1 recovered from fibroblasts transfected with helper virus DNA. DNAs in lanes 1, 3, 5, 6, 8 and 10 were digested with *Bam*HI. Hybridization probes used: lanes 1–5, probe C; lanes 6–10, probe D described in Fig. 1. Length of hybridized bands are given in kb; arrows indicate the bands derived from the parental or processed hybrid immunoglobulin gene.

Methods. The 8.2-kb *Sac*I fragment of pSNV₁·hV_{HBV}-mC_γ1 was isolated by agarose gel electrophoresis and ligated with T₄ DNA ligase at 15°C for 10 h. Homopolymeric SNV₁·hV_{HBV}-mC_γ1 and circular pSNV₂·mV_{T15}-hC_γ1 DNA (10 μg each) were introduced separately into chicken fibroblasts in a tissue-culture flask (25 cm²) in the presence or the absence of REV-A DNA (1 μg) by the calcium phosphate co-precipitation technique¹⁷. Virus production was assayed by the reverse transcriptase activity¹⁰. After 7–10 days, the tissue culture medium containing the recombinant virus was collected and fresh chicken cells in a 100-mm Petri dish were infected with undiluted virus. Three days after infection, non-integrated viral DNA was isolated by the Hirt procedure from the infected cells^{18,19}. The non-integrated viral DNAs were digested with *Bgl*II or *Bam*HI and analysed by electrophoresis in a 0.8% agarose gel. Each lane contained DNA from one 100-mm Petri dish. The gel was blotted onto nitrocellulose filter paper and the filter hybridized with the nick-translated ³²P-labelled probe indicated.



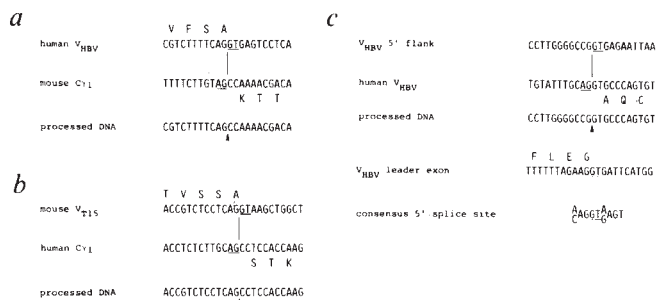


Fig. 3 Nucleotide sequences surrounding processed exon junctions in progeny viruses. **a**, Sequence surrounding the V_{HBV} and mouse C_{H1} exon junction in progeny virus of pSNV₁· V_{HBV} -mC_γ1. **b**, Sequence surrounding the V_{T15} and human C_{H1} exon junction in progeny virus of pSNV₂·mV_{T15}-hC_γ1. **c**, Sequence surrounding the recombination site between the potential donor splice site in the 5'-flanking region of the V_{HBV} gene and the V_{HBV} exon. Nucleotide sequences around the exon/intron junctions in parental virus are shown above the sequence of processed gene. The consensus sequence of the donor splice site¹² and the 5' splice site of leader sequence of V_{HBV} are also shown. GT and AG are underlined. Splice sites are designated by arrowheads. Nucleotide sequences determined (~200 bp at each junction) are identical to those predicted from parental gene sequences taken from the literature^{4,20,21}.

Method. Virus-infected chicken cells in 12 100-mm Petri dishes were lysed with SDS 3 days after infection. Non-integrated viral DNA was isolated by Hirt's procedure^{18,19}. The 1.7-kb *Bam*HI fragment containing the processed mV_{T15}-hC_γ1 gene and the 2.1-kb *Bgl*II fragment containing the processed hV_{HBV}-mC_γ1 gene were isolated by agarose gel electrophoresis. These fragments were inserted separately into Charon 28H, a modified Charon 28 vector (described below), with T₄ ligase and packaged *in vitro*. Recombinant phages containing hV_{HBV}-mC_γ1 and mV_{T15}-hC_γ1 were screened using probes A and C, respectively. We identified eight phage clones that contained the hybrid hV_{HBV}-mC_γ1 gene fragment and two phage clones that contained the hybrid mV_{T15}-hC_γ1 gene fragment. There is an *Eco*RI site in each arm of Charon 28H. We purified the *Eco*RI fragment containing processed hV_{HBV}-mC_γ1 gene and the *Bam*HI fragment containing processed mV_{T15}-hC_γ1 gene for restriction enzyme analysis and sequencing. The *Eco*RI fragment containing hV_{HBV}-mC_γ1 and the *Bam*HI fragment containing mV_{T15}-hC_γ1 were cleaved with restriction enzymes shown in Fig. 1 and their ends were labelled by polynucleotide kinase. Labelled fragments were isolated and sequenced according to Maxam and Gilbert²². We also sequenced the *Taq*I/*Xba*I fragment of the 5'-flanking region of the V_{HBV} gene. Construction of Charon 28H vector. The 6.8-kb *Bam*HI fragment of mouse immunoglobulin S_γ2a region gene²³ was cloned in pBR322. The recombinant plasmid was digested with *Bgl*II and inserted into *Bam*HI site of Charon 28 (ref. 24) to construct Charon 28H vector. Charon 28H DNA was digested with *Bam*HI and *Sal*I and the internal fragment derived from pBR322 was removed to prepare arms. Longer and shorter arms of the modified Charon 28H are 4.1 and 2.7 kb, respectively, longer than the original Charon 28.

there is preference of the 5' potential splice site over the normal donor site of the signal peptide exon. The nucleotide sequences of both the 5' potential and normal donor sites mismatch at two bases with the consensus 9-base sequence surrounding the donor splice site¹². Except for GGTGA sequence, there is little sequence homology between the nucleotide sequences surrounding the potential and normal donor sites. There are several examples where mutations in normal splice donor sites have revealed potential donor splice sites in exons¹³⁻¹⁶. The presence of a donor splice site more potent than the normal donor site in the flanking region has not been reported.

We have shown here that the strategy using the retrovirus vector is useful for construction of hybrid immunoglobulin genes and will eventually enable expression of the hybrid protein in yeast, *E. coli* and animal cells. Our results demonstrate that the genomic V and C exons derived from different species are properly fused. We have used the same strategy to construct the hybrid light-chain genes composed of mouse V_K and human C_K genes. Most clinically useful antibodies are made more easily in mouse and are difficult to administer in humans. If the C

region of the mouse monoclonal antibody against human cancer cells could be replaced by a human immunoglobulin C region, the clinical advantages would include the facts that the hybrid immunoglobulin is less antigenic and the most useful C_H region could be chosen for treatment.

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- Ritz, J. & Shlossman, S. F. *Blood* **59**, 1-11 (1982).
- Ritz, J. *et al. Blood* **58**, 141-152 (1981).
- Miller, R. A., Maloney, D. G., Warnke, R. & Levy, R. *New Engl. J. Med.* **306**, 517-522 (1982).
- Honjo, T. *et al. Cell* **18**, 559-568 (1979).
- Shimotohno, K. & Temin, H. M. *Cell* **26**, 67-77 (1981).
- Takahashi, N. *et al. Cell* **29**, 671-679 (1982).
- Nakanishi, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6984-6988 (1982).
- Shimotohno, K. & Temin, H. M. *Nature* **299**, 265-268 (1982).
- O'Rear, J. J. & Temin, H. M. *J. Virol.* **39**, 138-149 (1981).
- Hagino, K., Kano, K., Ikegami, S., Nagano, H. & Mano, Y. *Virology* **107**, 174-179 (1980).
- Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4853-4857 (1978).
- Cech, T. R. *Cell* **34**, 713-716 (1983).
- Seidman, J. G. & Leder, P. *Nature* **286**, 779-783 (1980).
- Choi, E., Kuehl, M. & Wall, R. *Nature* **286**, 776-779 (1980).
- Wieringa, B., Meyer, F., Reiser, J. & Weissmann, C. *Nature* **301**, 38-43 (1983).
- Treisman, R., Orkin, S. & Maniatis, T. *Nature* **302**, 591-596 (1983).
- Graham, F. L. & Van der Eb, A. J. *Virology* **52**, 456-467 (1973).
- Hirt, B. *J. molec. Biol.* **26**, 365-369 (1967).
- Chen, I. S. Y. & Temin, H. M. *J. Virol.* **41**, 183-191 (1982).
- Ellison, J. W., Berson, B. J. & Hood, L. E. *Nucleic Acids Res.* **10**, 4071-4079 (1982).
- Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. *Cell* **19**, 981-992 (1980).
- Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Nikaido, T., Yamawaki-Kataoka, Y. & Honjo, T. *J. biol. Chem.* **257**, 7322-7329 (1982).
- Liu, C.-P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. *Science* **209**, 1348-1353 (1980).

Thymic epithelium and the induction of transplantation tolerance in nude mice

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Immunological tolerance of self has been studied experimentally by the induction of unresponsiveness to antigens of the major histocompatibility complex (MHC) in neonatal mice¹. The specific unresponsiveness resulting from such neonatal tolerance induction is first demonstrable in the thymus^{2,3}, suggesting that neonatal tolerance is induced by some cellular component of the thymus, or at some prethymic stage. Recent transplantation studies suggest that thymic epithelium, derived by organ culturing fetal mouse thymus in the presence of deoxyguanosine, survives in an allogeneic host environment despite the continued expression of MHC donor antigens, but fails to induce allotolerance⁴. We demonstrate here that embryonic thymus lobes organ cultured at 24°C, a treatment that deletes the lymphohaematopoietic component of thymus leaving the epithelial matrix intact⁵, when transplanted to intact histoincompatible recipients, similarly survive for a prolonged period and do not induce tolerance to donor MHC antigens. However, when such culture-derived thymic epithelium is allografted to athymic nude mice, host-derived lymphocytes from both the epithelial graft and recipient spleen are unresponsive to the MHC antigens of the epithelial donor. The results suggest that, when investigated in a system which precludes the possible involvement of extrinsic mature T cells, processed by the syngeneic host thymus, thymic epithelium may induce transplantation tolerance.

Low-temperature (24°C) organ culture was first used to deplete passenger leukocytes in studies of pancreatic islet

Table 1 Effect of low-temperature organ culture on the survival of fetal thymus grafts in allogeneic recipients

Duration of grafts (days)	No. of animals with surviving grafts		
	Low-temperature culture (24 °C)	Conventional culture (37 °C)	Uncultured 14-day thymus
5	5/5	5/5	5/5
10	5/5	0/5	3/5
15	5/5	0/5	1/5
25	5/5	0/5	0/5
40	3/5	0/5	0/5
50	1/5	0/5	0/5

Fetal thymus lobes were isolated from C57BL/6 (H-2^b) embryos on day 14 of gestation and organ cultured for 8 days at either 24 or 37 °C as described previously⁶. Two lobes were transplanted beneath the renal capsule of C₃H (H-2^k) recipients. Uncultured lobes were transplanted to allogeneic recipients immediately following isolation from 14-day donor embryos. Host mice were killed at varying times post-grafting (5–50 days) and the presence or absence of grafts was scored using a dissecting microscope. Successful grafts were readily identified as fleshy, white subcapsular swellings. The absence of grafts was always confirmed histologically. Grafts of fetal thymus, whether low-temperature organ cultured or not, survived indefinitely in syngeneic recipients of allogeneic (H-2^d) nude mice (data not shown). The results show that, whilst untreated 14-day thymic lobes and those organ cultured at 37 °C are acutely rejected by allogeneic hosts, low-temperature (24 °C) cultured grafts survive for prolonged periods.

allograft survival⁶ and has been shown subsequently to delete selectively the lymphoid component of fetal mouse thymus, leaving the epithelial matrix intact^{5,7}. When 14-day fetal thymus lobes, cultured for 8 days at 24 °C, are grafted beneath the renal capsule of intact syngeneic mice, the surviving thymic epithelial matrix is colonized by host-derived lymphoid precursors which differentiate into thymocytes, thus forming an heterotopic lymphoid thymus^{5,8}. The regenerating intragraft lymphocyte population is 95% Thy-1⁺, 80% TL⁺, 95% Lyt 1⁺, 90% Lyt 2⁺ and includes a cortisone-resistant functional subpopulation responsive to alloantigens in mixed lymphocyte culture (MLC) as well as to T-cell mitogens (refs 8, 9 and R.K.J. and N.A.H., unpublished data).

It has been reported previously that embryonic thymus lobes depleted of their endogenous lymphohaematopoietic cells by deoxyguanosine treatment *in vitro* can be successfully transplanted across major histocompatibility barriers, even though the surviving thymic epithelium continues to express class II MHC antigens⁴. To determine whether low-temperature organ culture

would allow the similar survival of fetal thymic allografts, C3H (H-2^k) mice were grafted with thymus lobes from C57BL/6 (H-2^b) embryos of 14 days' gestation, organ cultured for 8 days at 24 °C. Survival of these experimental grafts was compared with that of 14-day fetal thymic lobes with and without conventional organ culture at 37 °C. Table 1 shows that, whereas uncultured and conventionally cultured grafts are rejected normally by 15 days post-grafting, fetal thymus lobes cultured at 24 °C escape this acute rejection. Survival of these thymic epithelial allografts, at least in this strain combination (C57BL/6 into C3H), is not indefinite. Although 100% of low-temperature treated grafts were recovered at 25 days, this proportion fell to 60% by 40 days and to 20% by 50 days. Surviving allogeneic epithelial grafts seem to grow in the same way as they do in syngeneic recipients, demonstrating organotypic thymic development and giving a maximal lymphoid cell yield of about 5×10^6 per lobe.

The probable explanation for the observed prolongation of allograft survival is that the low-temperature treatment of fetal thymus not only eliminates the endogenous thymocytes and their progenitors, but also substantially reduces or even deletes the donor class II MHC-antigen-expressing dendritic cells of the thymus, thereby avoiding route 1 rejection as described by Lechler and Batchelor¹⁰. Thus, when allografted, the thymic epithelium derived by low-temperature organ culture seems to behave in a similar fashion to that obtained by deoxyguanosine treatment⁴, except that, over the longer timescale reported here, low-temperature-treated grafts seem eventually rejected. This late rejection may reflect the survival of small numbers of donor-derived dendritic cells^{11,12} of haematogenous origin within the graft or it may be a manifestation of the alternative rejection route proposed by Lechler and Batchelor¹⁰ by which graft antigens are processed and presented by the recipients' own accessory cells.

We confirm the findings of Ready *et al.*⁴ that lymphocytes recovered from both the epithelial grafts and the spleens of intact allogeneic recipients respond to the MHC antigens of the thymic donor type in MLC (Table 2), arguing against a role for thymic epithelium in the induction of tolerance. However, as the recipients of the thymic epithelial grafts were normal intact histoincompatible mice, it cannot be concluded necessarily that the reactive T cells present in the grafts had, in fact, matured there. Indeed, it is possible that the reactive T cells had matured in the syngeneic recipient thymus and subsequently migrated into the allogeneic thymic epithelial graft. To eliminate this possibility, we also transplanted low-temperature organ-culture-derived thymic epithelium to athymic nude mice, where there was no possibility of host thymic processing.

Athymic BALB/c *nu/nu* (H-2^d) mice were grafted with low-temperature-treated C57BL/6 (H-2^b) fetal thymic lobes. Such

Table 2 MLC responses of spleen and thymus graft cells from C₃H(H-2^k) mice bearing low-temperature organ-cultured C57BL/6 (H-2^b) thymus grafts beneath the renal capsule

Animal no.	Responders	Stimulators			P values
		Host type (H-2 ^k)	Graft type (H-2 ^b)	Third party (H-2 ^d)	
1	Intragraft lymphocytes	564 ± 91	4,815 ± 736	5,392 ± 124	<0.01; <0.001
	Host spleen	16,006 ± 1,079	38,048 ± 312	49,546 ± 1,318	<0.001; <0.001
2	Intragraft lymphocytes	475 ± 40	2,368 ± 178	1,517 ± 231	<0.001; <0.05
	Host spleen	7,807 ± 673	18,780 ± 52	24,250 ± 295	<0.001; <0.001
3	Intragraft lymphocytes	362 ± 40	3,364 ± 143	2,454 ± 155	<0.001; <0.001
	Host spleen	NT	NT	NT	

MLC assays were performed in U-well microtitre plates. Responder cells were prepared by teasing host spleen and thymus grafts and were used at a final concentration of 2×10^5 per well. Stimulators were mitomycin-C-treated ($30 \mu\text{g ml}^{-1}$ for 40 min at 37 °C) spleen cells titrated over the range 4×10^5 – 2.5×10^4 per well. All cultures were performed in 200 μl of supplemented RPMI 1640 medium with 10% fetal calf serum. Cultures were pulsed with $1 \mu\text{Ci ml}^{-1}$ of ³H-thymidine for 18 h on day 4 of culture and collected with an automatic sample collector. Results are expressed as the mean d.p.m. ± s.e.m. for three replicate wells at the optimal stimulator: responder ratio. P values are given for the response to graft-type and third-party stimulators as compared with the response to host-type stimulators. The results show that there are significant responses to both graft-type and third-party stimulators by cells from both host spleen and thymus grafts. NT, not tested.

Table 3 MLC responses of spleen and thymus graft cells from athymic BALB/c (H-2^d) nude mice bearing low-temperature organ culture C57BL/6 (H-2^b) thymus grafts beneath the renal capsule

Animal no.	Responders	Stimulators			P values
		Host type (H-2 ^d)	Graft type (H-2 ^b)	Third party (H-2 ^k)	
1	Intragraft lymphocytes	412 ± 95	326 ± 102	4,338 ± 59	NS; <0.001
	Host spleen	12,261 ± 1,471	16,672 ± 896	25,614 ± 2,348	NS; <0.01
2	Intragraft lymphocytes	258 ± 60	243 ± 53	2,779 ± 159	NS; <0.001
	Host spleen	13,212 ± 1,294	14,417 ± 796	24,212 ± 1,261	NS; <0.01
3	Intragraft lymphocytes	468 ± 114	859 ± 246	2,050 ± 244	NS; <0.01
	Host spleen	16,438 ± 2,615	17,046 ± 1,050	28,793 ± 2,150	NS; <0.01

MLC cultures were carried out as described in Table 2 legend. The results show that while there are significant responses to third-party stimulators by cells from both nude host spleen and thymus grafts, both populations failed to respond to graft-type stimulators. NS, not significant.

thymic epithelial grafts, placed beneath the renal capsule, became recolonized by T-cell precursors to form a fully lymphoid heterotopic thymus. The host derivation of the repopulating lymphoid component was confirmed using monoclonal anti-D^d (supernatant from clone 34-4-21s) and anti-K^b (supernatant from clone 28-13-3s) antibodies (data not shown). By 56 days post-grafting, the peripheral T-cell compartment of the nude recipients was significantly reconstituted as judged by the number of Thy-1⁺ cells in the host spleen and the restoration of responsiveness to the T-cell mitogen concanavalin A (Con A) (Table 4). In these nude recipients, we found that host-derived lymphocytes (H-2^d) within the epithelial graft respond in MLC (Table 3) to the MHC antigens of a third party (H-2^k), but not to the MHC antigens of the thymic epithelial donor (H-2^b). Table 3 also shows that spleen cells from the recipient nudes respond to third-party stimulators (H-2^k) but not to graft type (H-2^b); however, the high level of proliferation to host type (H-2^d) obscures a clear demonstration of either reconstitution of the MLC response or tolerance in the spleen population.

Thus, in the nude thymic chimaera, the low-temperature-cultured thymic allografts can be shown to induce tolerance while failing to do so in normal thymus-intact recipients. The simplest explanation for this paradox is that the T cells reactive to donor graft type in the intact chimaera had matured in the syngeneic host thymus and migrated to the allogeneic thymic graft. That mature T cells can indeed recirculate to the normal thymus has been suggested previously by migration studies with labelled effector T-lymphocyte lines¹³. The results suggest that the epithelium remaining in low-temperature cultured grafts can induce tolerance, but that in allogeneic normal recipients this tolerance is masked.

That it is the grafted thymic epithelium which leads to tolerance induction is at this time only a probability, for the involvement of thymic dendritic cells cannot be entirely ruled out. We have been unable to isolate dendritic cells from low-temperature thymic organ cultures before grafting⁸, but it cannot be pre-

cluded that the low-temperature treatment only reduces the number of dendritic cells rather than eliminating them altogether. However, as low-temperature-treated thymic allografts survive in thymus-intact recipients long after the stage of acute rejection, the presence of functionally significant numbers of dendritic cells is doubtful.

Although there is little question that the tolerance to donor MHC antigens observed in both the grafts and spleens of the nude recipients was induced by the presence of the low-temperature organ-cultured thymic lobes, the present study sheds little light on the nature of the tolerogenic mechanism. While these results are in keeping with the hypothesis that tolerance results from the deletion of clones reactive to graft type², the unresponsiveness to donor MHC antigens could be mediated by specific suppressor T cells, as demonstrated for classical neonatal tolerance^{14,15}.

In conclusion, we suggest that it is premature to exclude thymic epithelium from having a determining effect in tolerance induction, as our results can be interpreted as suggesting that thymic epithelial cells, which are known to express class II MHC antigens^{16,17}, may have a role in the induction of tolerance in helper T lymphocytes. Whether cultured thymic epithelium tolerizes cytotoxic T lymphocytes remains to be determined, but in this context it is interesting that the thymus appears to impose MHC restriction only for class II- and not for class I-restricted T-cell recognition^{18,19}.

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Note added in proof: It has recently been shown that thymocytes from deoxyguanosine-treated fetal thymi grafted to allogeneic nude recipients contain cytolytic T-lymphocyte precursors reactive to class I MHC antigens of thymic donor type²⁰.

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Table 4 Thy-1 expression and Con A responsiveness of spleen lymphocytes recovered from BALB/c nude mice with and without C57BL/6 thymic epithelial grafts

Recipient	Graft	No. Thy 1.2 (CI ± s.e.m.)	Con A (̄Δ d.p.m. ± s.e.m.)
nu/+ (27)	—	28.2 ± 4.2	61,067 ± 6,427
nu/nu (12)	—	0.1 ± 0.6	370 ± 1,354
nu/nu (10)	+	9.6 ± 1.5	33,118 ± 6,922

CI, cytotoxic index, using anti-mouse Thy 1.2 (clone 30-H12, Becton-Dickinson) at 1 in 800 and rabbit complement (Low Tox M, Cedarlane) at 1 in 15. Viability was assessed by trypan blue dye exclusion and a total of 400 cells counted. For Con A experiments, 200-μl microcultures in triplicate using 2 × 10⁵ spleen cells and 2 μg ml⁻¹ Con A (Miles-Yeda) were incubated for 72 h with a pulse of 1 μCi ml⁻¹ for the final 18 h. Numbers in parentheses are the number of mice tested.

1. Billingham, R. E., Brent, L. & Medawar, P. B. *Nature* **172**, 603-606 (1953).
2. Nossal, G. J. V. & Pike, B. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3844-3847 (1981).
3. Wood, P. J. & Streilein, J. W. *Eur. J. Immun.* **12**, 188-194 (1982).
4. Ready, A. J., Jenkinson, E. J., Kingston, R. & Owen, J. J. T. *Nature* **310**, 231-233 (1984).
5. Jordan, R. K. & Crouse, D. A. in *Development and Differentiation of Vertebrate Lymphocytes* (ed. Horton, J. D.) 47-61 (Elsevier, Amsterdam, 1980).
6. Lacy, P. E., Davie, J. M. & Finke, E. H. *Science* **204**, 312-313 (1979).
7. Robinson, J. H. & Jordan, R. K. *Immun. Today* **4**, 41-45 (1983).
8. Jordan, R. K., Bentley, A. L., Perry, G. A. & Crouse, D. A. *J. Immun.* (in the press).
9. Jordan, R. K., Robinson, J. H., Bentley, A. L., House, K. C. & Hopkinson, N. A. in *Proc. 8th int. Conf. Lymphatic Tissues and Germinal Centers in Immune Reactions* (ed. Klaus, G. G. B.) (Plenum, New York, in the press).
10. Lechler, R. I. & Batchelor, J. R. *J. exp. Med.* **155**, 31-41 (1982).
11. Robinson, J. H. *J. Immun.* **130**, 1592-1595 (1983).
12. Barclay, A. N. & Mayrhofer, G. J. *J. exp. Med.* **153**, 1660-1671 (1981).
13. Fink, P. J., Bevan, M. J. & Weissman, I. L. *J. exp. Med.* **159**, 436-451 (1984).
14. Dorsch, S. & Roser, B. *Transplantation* **33**, 518-524 (1982).
15. Stockinger, B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 220-223 (1984).
16. Natali, P. G., de Martino, C., Pellegrino, M. A. & Ferrone, S. *Scand. J. Immun.* **13**, 541-546 (1981).
17. Jenkinson, E. J., van Ewijk, W. & Owen, J. J. T. *J. exp. Med.* **153**, 280-292 (1981).
18. Bradley, S. M., Kruisbeek, A. M. & Singer, A. *J. exp. Med.* **156**, 1650-1664 (1982).
19. Kast, W. M., de Waal, L. P. & Melief, J. M. *J. exp. Med.* **160**, 1752-1766 (1984).
20. von Boehmer, H. & Schubiger, K. *Eur. J. Immun.* **14**, 1048-1052 (1984).

Activation of major histocompatibility complex class I mRNA containing an *Alu*-like repeat in polyoma virus-transformed rat cells

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Class I genes of the major histocompatibility complex (MHC) appear to be activated in mouse cells transformed by the DNA tumour virus simian virus 40 (SV40)¹. Conversely, suppression of MHC class I genes has been reported in adenovirus-12-transformed baby kidney rat cells². We have now investigated the expression of genes encoded by the rat MHC locus in rat fibroblast cells transformed by polyoma virus (Py). Using a mouse genomic *H-2* clone as a probe in Northern transfer hybridization analysis, we have observed a high level of expression of rat MHC class I messenger RNA in all the transformed rat cell lines analysed. The class I 1.6-kilobase (kb) mRNA activated in Py-transformed rat cells appears to contain an *Alu*-like type II repeat element, as the same 1.6-kb mRNA is detected using either the *H-2* class I sequence or a repetitive *Alu*-like type II element as a probe. High levels of heterogeneous poly(A)⁺ transcripts of 0.5–0.8 kb are also observed in Py-transformed rat cells using probes containing an *Alu*-like type II repetitive element.

The rat major histocompatibility complex, the *RT.1* complex, comprises the loci encoding the histocompatibility antigens³. Three loci, *RT.1 A* (analogues of mouse *H-2K* and *H-2D*)⁴, *RT.1 E* (ref. 5) and *RT.1 C* (*Qa* analogue)⁶, control the expression of the classical serologically defined class I antigens. Taking advantage of the relatively conserved nature of portions of mammalian MHC genes, we have used a mouse genomic *H-2* DNA probe to investigate the level of *RT.1* class I mRNA expression in Py-transformed rat cells and the parental untrans-

formed cells. Figure 1 describes the structure of the mouse *H-2* clone pL4-7. The 2.7-kb *Bam*HI fragment isolated from a genomic library constructed from mouse DBA/2 seems to be similar, if not identical, to the 3'-end portion of the 27.1 gene isolated by Steinmetz *et al.*⁷ from a BALB/c DNA library. As the probe contains the constant (third) domain, it should react with any MHC class I heavy-chain mRNA present in rat cells.

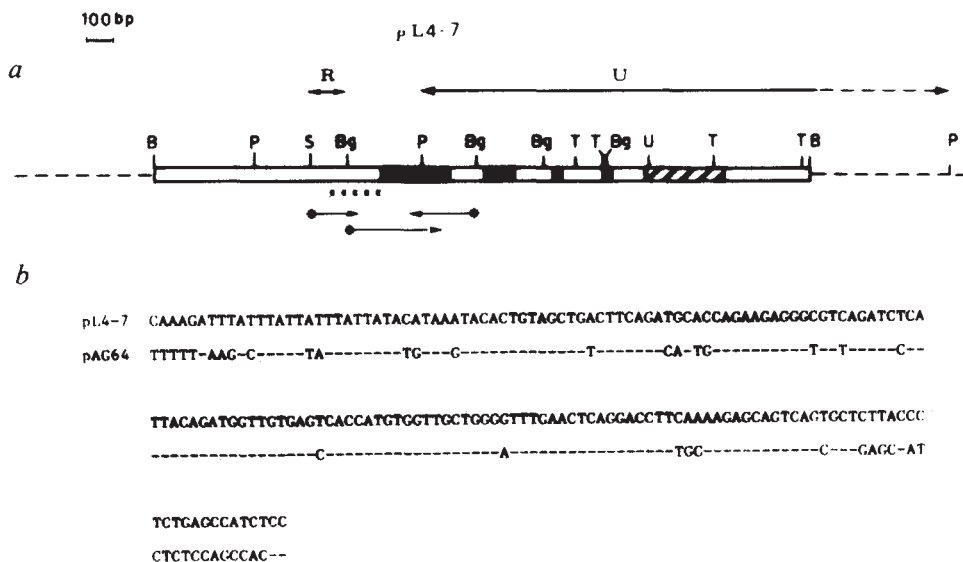
A series of Py-transformed rat cell lines was used to demonstrate the general applicability of the observed phenomena (Table 1). The Rat-1 (ref. 8) parental cell line and the Ag4-rat revertant cell line R3-rat, which has lost the integrated Py early DNA coding sequence (G.L.M. and L.L., unpublished results), were used as untransformed control cells.

Polyadenylated cytoplasmic RNAs from parental, transformed and revertant normal cells were electrophoresed in formaldehyde/agarose gel, transferred to nitrocellulose filter and hybridized with ³²P-labelled pL4-7 plasmid DNA (Fig. 2). All the Py-transformed rat cells analysed (Table 1), regardless of their origin, expressed a high level of mRNA of 1.6 kb and a heterogeneous class of transcripts of 0.5–0.8 kb (Fig. 2 and data not shown). The various Py-rat cell lines analysed seemed to express slightly different patterns of the heterogeneous small RNAs. Conversely, the untransformed parental Rat-1 and the normal revertant cells (Fig. 2, lanes 1, 2) expressed a very low level of 1.6-kb mRNA, which was almost undetectable after long exposure of these autoradiograms (2 weeks at –80 °C). The heterogeneous class of RNA transcripts of 0.5–0.8 kb was observed in the normal cells, although at a lower level in Rat-1 cells. Attempts to resolve the hybridization signal observed at 0.5–0.8 kb into discrete bands using different gel concentrations and electrophoresis conditions were unsuccessful.

These results indicate that a high level of expression of MHC class I mRNA is a common characteristic of Py-transformed rat cells, extending to the Py-rat system the notion originally proposed¹ that activation of class I major histocompatibility antigen genes is a general feature of oncogenesis in the mouse. However, the activation of MHC class I gene expression in Py-rat does not appear to be a general phenomenon of transformed rat cells, as it has been reported^{2,9} that a MHC gene, *RT.1 A*^u, is switched off in adenovirus-12-transformed primary baby rat kidney cells.

To rule out the possibility that the high level of expression of *RT.1* class I mRNA in Py-rat cells is a result of extensive amplification of the corresponding *RT.1* genomic sequences, we have used the Southern¹⁰ hybridization technique to analyse the structure of the *RT.1* locus in Py-rat and in the normal

Fig. 1 *a*, Map of pL4-7. This plasmid was isolated originally by A. M. Frischauf, (EMBL) from DBA/2 mouse DNA. Fine restriction mapping and partial DNA sequencing have revealed that the pL4-7 is similar to the 3'-end portion of the 27.1 clone isolated by Steinmetz *et al.*⁷. Black boxes indicate the positions of the exons, a hatched box denotes the 3'-untranslated region as indicated by Steinmetz *et al.*⁷. Partial DNA sequencing was performed across exon 4 and we found no difference from the reported sequence of the 27.1 clone. The arrows indicate the direction and length of the sequences obtained by the chemical degradation method²⁷. « « « Indicates the position of an *Alu*-like type II repeat. Subclone 'R' was constructed by inserting the 137-bp *Sall*/*Bgl*III fragment in the *Bam*HI/*Sall* site of plasmid pAT153 (ref. 28). The *Sall*/*Bgl*III fragment comprises 72 bp of the repetitive element and 65 bp of the adjacent intron sequences as determined by DNA sequencing. Subclone 'U', containing the 3'-end coding region devoid of the repetitive element, was constructed by subcloning the 2.7-kb *Pst*I fragment in the *Pst*I site of pAT153. B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; S, *Sall*; T, *Sst*I; U, *Pvu*II. *b*, Comparison of the *Alu*-like repeat present in the pL4-7 and the repetitive element in the plasmid pAG64¹ written in the opposite orientation. –, Identical sequences.



Rat-1. No difference was detected between the Rat-1 and Py-rat cell DNAs (data not shown).

The presence of a type II *Alu*-like repeat within pL4-7 (Fig. 1) raises the question of the relationship between the mRNAs found in the Py-rat and the repetitive element. To answer this question, we have constructed subclones of pL4-7 containing either the repetitive element (subclone 'R'; see Fig. 1) or exclusively the non-repetitive *H-2* sequence (subclone 'U'; see Fig. 1), and have used these as probes in Northern transfer experiments. Figure 3 shows that probe R detects all the RNA transcripts observed with the pL4-7, whereas probe U detects only the 1.6-kb mRNA. These data indicate that the heterogeneous RNA transcripts of 0.5–0.8 kb hybridize only to *Alu*-like repeat, whereas the 1.6-kb mRNA is composed of the *RT.1* class I sequence containing an *Alu*-like type II repeat.

The activation of a MHC class I gene containing a repetitive element has been documented by Rigby's group^{1,11,12} in SV40-transformed mouse cells and in two Py-mouse cell lines, where they have characterized sets of genes silent in most normal cells but active in oncogenic transformed mouse cells. The prototype complementary DNA clone of Set 1, pAG64 (ref. 1), encodes a *H-2* class I antigen and contains a dispersed repetitive element in the 3'-untranslated region of the mRNA which is very similar to the repetitive element located in the opposite orientation within one of the introns in pL4-7 (see Fig. 1b). Both these *Alu*-like elements are members of the *B2* family originally defined by hybridization to 'fold-back' heterogeneous nuclear RNA^{13,14}. The *Alu*-like repeat in pL4-7 diverges 14% from the pAG64 repeat and 12% from the consensus *B2* family sequence¹³.

Alu-like type II repetitive elements have been found in the 3'-untranslated region of cDNA clones encoded in the *H-2D* and *H-2L* regions¹⁵⁻¹⁹, whereas cDNA clones encoding the *K^b* (ref. 20) and *K^d* (ref. 21) antigens lack repetitive sequences. The presence of *Alu*-like repeats in both mouse and rat MHC class I mRNAs may reflect a similar evolutionary history of their MHC.

Several conclusions can be drawn from our work. First, the elevated level of 1.6-kb mRNA appears to be independent of the expression of Py early large T-antigen [relative molecular mass (*M_r*) 100,000 (100K)] and small T-antigen (*M_r* 22K) (Table 1, Fig. 2), indicating that the presence of the 55K middle T-antigen is sufficient to induce a high level of 1.6-kb mRNA. As the 55K middle T-antigen is a non-nuclear protein and as there is no evidence that it can regulate gene transcription directly, one must postulate the presence of cell-encoded factor(s) involved in the activation of MHC class I mRNA in response to viral transformation. Second, the activation of 1.6-kb mRNA appears to be a reversible phenomenon, as low levels of 1.6-kb

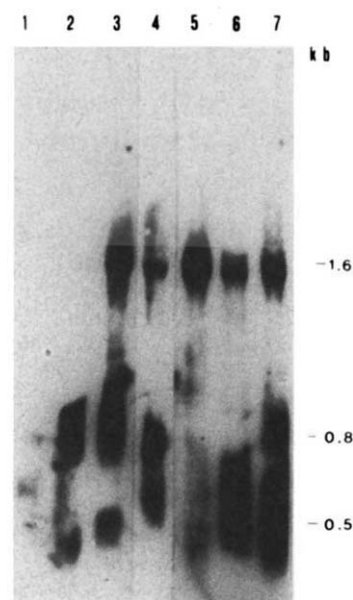


Fig. 2 RNA transfer hybridization analysis of normal and transformed rat cell lines. Polyadenylated cytoplasmic RNA was isolated as described previously²⁹. Aliquots (2 µg) of poly(A)⁺ RNA (selected once) were fractionated by electrophoresis in 1.3% agarose/formaldehyde gels and transferred to nitrocellulose filters³⁰. The blots were heated for 2 h at 80 °C and prehybridized for 5 h at 42 °C in a buffer containing 5× Denhardt's solution (0.1% each of Ficoll, bovine serum albumin and polyvinylpyrrolidone), 0.25 mg denatured calf thymus DNA per ml and 50% formamide. Subsequently, the filters were hybridized at 42 °C in the same buffer containing 20 ng ml⁻¹ ³²P-labelled pL4-7 DNA. The pL4-7 DNA was labelled by nick-translation³¹ at a specific activity of 2 × 10⁸ c.p.m. µg⁻¹. After hybridization, the filters were washed three times in 2×SSC, 0.1% SDS at room temperature, then at 50 °C for ~2 h. The filters were then autoradiographed at -80 °C with intensifying screen for 72 h. RNA samples are: lane 1, Rat-1; lane 2, R3-rat; lane 3, 7ax; lane 4, 7axT; lane 5, 82-rat; lane 6, Ag4-rat; lane 7, MT-rat. The gels were routinely calibrated with ribosomal RNA of various sources and with ³²P-labelled DNA plasmid size markers (×10⁻³).

mRNA are found in the revertant cell line R3-rat. Third, elevated levels of 1.6-kb mRNA are found both in *in vitro* Py-rat cells and in cell lines established from tumours induced with Py-rat cells. Fourth, it appears that in neoplastic mouse and rat cells transformed by two similar oncogenic viruses, SV40 and Py, the activation of MHC class I mRNA containing a similar repetitive

Table 1 Properties of the polyoma-transformed rat cell lines used

Cell lines	Ref.	T-antigen species				Isolation		
		Large-T	Middle-T	Small-T	Others	Foci	Agar	Tumour
7ax	22	+	+	+	—	+		
82-rat	22	—	+	+	63K, 40K, 32K	+		
53-rat	22	—	+	+	—	+		
H-2	23	+	+	+	—	+		
Ag4-rat		—	+	+	70K		+	
MT-rat		—	+	—	—	+		
7axT	8	—	+	+	75K			+
7axB	24	+	+	+	—			+
8aT	25	—	+	+	35K			+
82T	25	—	+	+	63, 40K, 32K			+
53T	25	—	+	+	—			+

The cell line MT-rat was isolated and characterized after DNA transfection of plasmid pPY-MT²⁶ on Rat-1 cells. The MT-rat cells contain about three copies of integrated Py DNA sequences, and express only the 55K middle T-antigen, as determined by immunoprecipitation with anti-T serum (L.L., unpublished results). The Ag4-rat cell line was isolated using a polyoma mutant Py9-1 which has lost part of the polyoma DNA sequences unique to the 100K large T-antigen coding sequences, but retains the ability to express middle and small T-antigens. The Ag4-rat cells express the 22K, 55K and 70K T-antigens, as determined by immunoprecipitation with anti-T serum (G.L.M. and L.L., unpublished results).

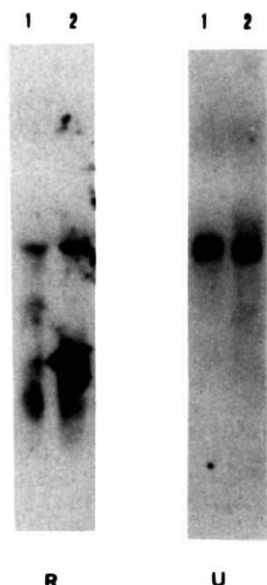


Fig. 3 Northern hybridization analysis of activated transcripts using the 'R' and 'U' probes (Fig. 1). Cytoplasmic poly(A)⁺ RNA extracted from 7ax cells (lane 1) and 7axT (lane 2) were fractionated in 1.3% agarose/formaldehyde gels, then processed as described in Fig. 2 legend. Aliquots (5 µg) of poly(A)⁺ RNAs were used. The exposure time was 1 week at -80 °C for blot 'R' and 48 h for blot 'U'.

element takes place. However, until the mRNA activated in the Py-transformed rat cells has been cloned, the homologous MHC class I genes activated in the mouse and rat transformed cells remain undefined.

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- Brickell, P. M., Latchman, D. S., Murphy, D., Willinson, K. & Rigby, P. W. J. *Nature* **306**, 756-760 (1983).
- Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. *Nature* **305**, 771-775 (1983).
- Gill, T. J., Cramer, D. V. & Kunz, H. W. *Am. J. Path.* **90**, 735-778 (1978).
- Gunther, E. & Stark, O. *The Organization of the Major Histocompatibility Systems in Man and Animals* (ed. Gotze, D.) 207-253 (Springer, New York, 1977).
- Kunz, H. W., Gill, T. J. & Mistra, D. N. *J. Immun.* **128**, 402-408 (1982).
- Stock, W. & Gunther, E. *J. Immun.* **128**, 1923-1928 (1982).
- Steinmetz, M. *et al. Cell* **25**, 683-692 (1981).
- Lania, L., Hayday, A., Bjursell, G., Gandini-Attardi, D. & Fried, M. *Cold Spring Harb. Symp. quant. Biol.* **44**, 597-603 (1980).
- Bernards, R. *et al. Nature* **305**, 776-779 (1983).
- Southern, E. J. *molec. Biol.* **98**, 503-515 (1975).
- Scott, M. R. D., Westphal, K.-H. & Rigby, P. W. J. *Cell* **34**, 557-567 (1983).
- Murphy, D., Brickell, P. M., Latchman, D. S., Willinson, K. & Rigby, P. W. J. *Cell* **35**, 865-871 (1983).
- Krayer, A. S. *et al. Nucleic Acids Res.* **10**, 7461-7475 (1982).
- Kramarov, D. A., Lekakh, I. V., Samarina, O. P. & Ryskov, A. P. *Nucleic Acids Res.* **10**, 7477-7491 (1982).
- Hood, L., Steinmetz, M. & Malissen, B. A. *Rev. Immun.* **1**, 568-594 (1983).
- Steinmetz, M. *et al. Cell* **24**, 125-134 (1981).
- Lalanne, J. L. *et al. Nucleic Acids Res.* **10**, 1039-1049 (1982).
- Reyes, A. A., Schold, M., Itakura, K. & Wallace, R. B. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3270-3274 (1982).
- Moore, K. W., Sher, B. T., Sun, Y. H., Eakle, K. & Hood, L. *Science* **215**, 679-682 (1982).
- Reyes, A. A., Schold, M. & Wallace, R. B. *Immunogenetics* **16**, 1-9 (1982).
- Xin, J. H., Kvist, S. & Dobberstein, B. *EMBO J.* **1**, 467-471 (1982).
- Lania, L. *et al. Virology* **101**, 217-232 (1980).
- Fried, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2117-2121 (1983).
- Hayday, A., Ruley, H. E. & Fried, M. *J. Virol.* **44**, 67-77 (1982).
- Lania, L., Hayday, A. & Fried, M. *J. Virol.* **39**, 422-431 (1981).
- Treisman, R., Novak, U., Favalaro, J. & Kamen, R. *Nature* **292**, 595-600 (1981).
- Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Twigg, A. J. & Sherratt, D. *Nature* **283**, 216-218 (1980).
- Favalaro, J., Treisman, R. & Kamen, R. *Meth. Enzym.* **65**, 718-749 (1980).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
- Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-252 (1977).

Keratinocytes blocked in phorbol ester-responsive early stage of terminal differentiation by sarcoma viruses

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It has been suggested that the initiation step in mouse skin carcinogenesis involves an alteration in epidermal differentiation, as mouse basal keratinocytes exposed to initiators resist the arrest of cell growth that is normally associated with the induction of terminal differentiation by calcium ions¹⁻³. The growth of epidermal basal cells infected by Kirsten (Ki) or Harvey (Ha) sarcoma viruses is, however, arrested in response to calcium ions, although the cells do not progress through their entire maturation programme when a functioning *ras* gene of those viruses is expressed⁴. If continuous proliferation in the differentiating cell layers is a requirement for tumour formation in skin^{3,5}, the response of sarcoma virus-infected cells seems inconsistent with the suggestion that an activated *ras* gene is sufficient to initiate skin carcinogenesis⁶. We now show that sarcoma virus-infected keratinocytes, when induced to differentiate, are blocked at an early, reversible stage of maturation. Furthermore, the cells respond to phorbol ester tumour promoters by undergoing a phenotypic reversion to a less mature stage. These results suggest that activation of a *ras* gene can produce conditionally initiated cells, in which the full expression of tumorigenicity depends on exposure to tumour promoters.

Mouse basal keratinocytes can be grown selectively in <0.1 mM Ca²⁺ medium (low Ca²⁺) and induced to stop growing, differentiate terminally and slough from the culture dish by >0.1 mM Ca²⁺ medium (high Ca²⁺)⁷. (By previously published criteria for biological responses, any Ca²⁺ concentration <0.1 mM is considered low Ca²⁺ and >0.1 mM is considered high Ca²⁺.) Carcinogen treatment of basal cells produces foci that continue to proliferate when challenged with high Ca²⁺ medium. Such studies have formed the basis for the hypothesis that this cellular change represents the initiation event^{1-3,8}. It seems likely that cells that have undergone the initiation step possess all the information required to produce papillomas^{3,5}. Recently, Balmain *et al.* have reported that Ha-*ras* expression is increased in epidermal papillomas and carcinomas⁶ and that more than 60% of papillomas contain an activated *ras* gene when tested by 3T3 transfection studies⁹. However, we have been unable to detect elevated levels of *ras* messenger RNA or other mRNAs hybridizable by complementary DNAs for viral oncogenes in putatively initiated epidermal cell lines²⁹. Furthermore, studies with sarcoma viruses indicate that an activated *ras* gene produces changes in keratinocytes which differ from those seen in putative chemically initiated cells and which are inconsistent with the expected requirements for papilloma formation^{3,4}. To clarify this discrepancy, we made further studies of the properties of keratinocytes expressing an activated Ha-*ras* and Ki-*ras* gene that had been introduced by virus infection.

Two epidermal marker proteins were examined, the pemphigoid and pemphigus vulgaris antigens. Pemphigoid antigen is a protein of relative molecular mass (*M_r*) 220,000 (220K) found in the basement membrane and it is closely associated with the plasma membrane of the basal cell^{10,11}. Pemphigus vulgaris antigen is a 210K glycoprotein containing disulphide-linked chains of 130K and 80K which occurs on the surface of stratifying epithelial cells¹². Immunofluorescence and immunoprecipitation data have shown that the pemphigoid antigen is synthesized exclusively by basal cells in low Ca²⁺; within 6 h of switch to high Ca²⁺, pemphigoid synthesis is

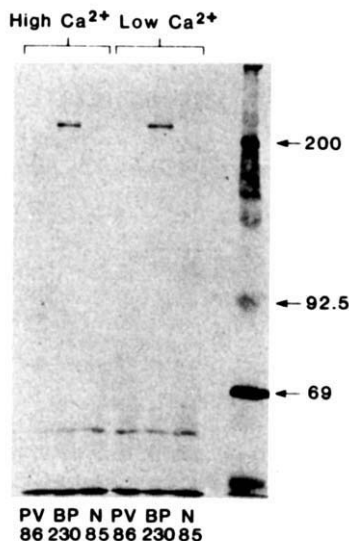


Fig. 1 Synthesis of epidermal antigens by virus-infected and control cells. Approximately 3×10^6 c.p.m. of each sample was immunoprecipitated with specific antisera (N, normal human serum; PV, pemphigus vulgaris antiserum no. 86; BP, bullous pemphigoid antiserum no. 230) and precipitates separated on a SDS-dithiothreitol (DTT) 6% polyacrylamide gel as reported previously¹². Fluorograms were developed after 3 weeks of exposure. Numbers refer to M_r markers ($\times 10^{-3}$). Only virus-infected cultures are shown.

Methods. Newborn BALB/c mouse keratinocytes were isolated and cultured in Eagle's medium with 8% Chelex-treated fetal calf serum (Reheis), 1% antibiotic/antimycotic mixture (Gibco) and 0.05 mM Ca^{2+} as described previously⁷. On day 3 *in vitro*, cultures were exposed to a virus complex containing Friend murine leukaemia virus (F-MuLV) clone 57 and Kirsten sarcoma virus in the presence of $4 \mu\text{g ml}^{-1}$ polybrene as reported previously⁴. Viral stocks were titred by xc plaque formation in NIH 3T3 cells⁴. Cells were exposed to 1:2 dilution of virus stock (multiplicity of infection of ~ 0.5) for 1 h and a dilution eightfold further was continued for 72 h. Fresh medium (0.05 mM Ca^{2+}) was added for an additional 24 h after which half of the dishes were switched to medium containing 1.2 mM Ca^{2+} . Cultures were maintained in either 1.2 mM or 0.05 mM Ca^{2+} for 48 h of which the final 16 h included $12.5 \mu\text{Ci ml}^{-1}$ ^{14}C amino-acid mixture (NEN, 55 mCi per mol atom carbon). The cell layer was extracted with 3 ml of 0.5% Nonidet P-40 in Tris-buffered saline (10 mM Tris HCl, pH 7.4, 0.15 mM NaCl) with 1 mM phenylmethylsulphonyl fluoride. After centrifugation at 100,000g for 1 h, the supernatant was dialysed against 0.3% Nonidet P-40 in Tris-buffered saline.

inhibited by $>90\%$ ¹³. Similar studies have indicated that pemphigus vulgaris antigen is synthesized exclusively by differentiating cells in high Ca^{2+} (ref. 13). Therefore, we prepared cell lysates from virus-infected keratinocytes that had been labelled with ^{14}C -amino acids for 16 h in low or high Ca^{2+} medium, and examined them by gel separation of immunoprecipitates using specific antisera to pemphigus vulgaris or pemphigoid antigens (Fig. 1). We found that, as occurs with normal cells, pemphigoid antigen was synthesized by infected keratinocytes in low Ca^{2+} , but unlike normal cells, synthesis was unchanged in infected cells maintained in high Ca^{2+} for 48 h. Furthermore, the extremely low levels of labelled pemphigus vulgaris antigen, expected in lysates from low Ca^{2+} cells, were also observed in lysates of virus-infected cells grown in high Ca^{2+} medium, culture conditions which stimulate pemphigus vulgaris synthesis in normal cells. Because the expression of these proteins is strongly linked to the differentiation state of keratinocytes¹³, the results suggested that virus-infected cells in high Ca^{2+} maintain basal cell properties.

Additional support for an early differentiative block of virus-treated cells was obtained in experiments in which cells were returned to low Ca^{2+} growth conditions after a period in high Ca^{2+} . Previously, Hennings and Holbrook¹⁴ had shown that

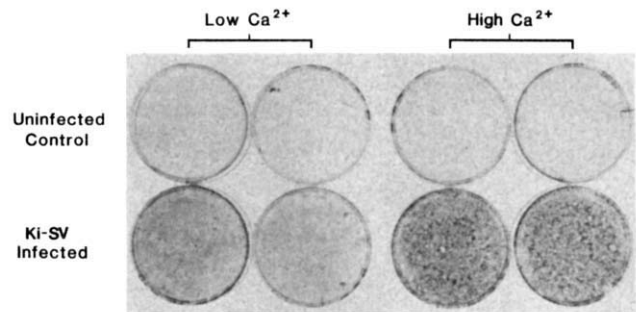


Fig. 2 Reversibility of the differentiation state of sarcoma virus-infected keratinocytes. Newborn BALB/c keratinocytes were isolated, plated and exposed to Kirsten virus as described in Fig. 1 legend. Five days after the start of virus exposure, all cultures were switched from 0.05 mM Ca^{2+} medium to 0.5 mM Ca^{2+} medium and maintained for 2 weeks with twice-weekly medium changes. Control and virus-infected dishes were then either switched back to 0.05 mM Ca^{2+} or continued in 0.5 mM Ca^{2+} , both for an additional 2 weeks. All dishes were then fixed in 10% formalin and stained with 10% Giemsa stain. Uninfected control cultures were exposed to polybrene only. Previously, controls exposed to polybrene only were shown to be identical to F-MuLV-exposed controls⁴. The low Ca^{2+} (0.05 mM) or high Ca^{2+} (0.5 mM) groups refer to the Ca^{2+} level of the final growth medium. Virus-treated cultures in 0.05 mM Ca^{2+} are not as dense as those in 0.5 mM Ca^{2+} and therefore do not stain as darkly, although both culture groups form completely confluent monolayers. Both uninfected control groups contained very few attached cells and thus show little staining.

keratinocytes are irreversibly committed to terminal differentiation by 72 h treatment in high Ca^{2+} . Such cells slough from culture even if switched back to the lower Ca^{2+} . This result is reproduced (Fig. 2) in uninfected control cultures, which were devoid of keratinocytes when maintained in high Ca^{2+} continuously for 4 weeks or when grown in high Ca^{2+} for 2 weeks and then returned to low Ca^{2+} for 2 weeks. The virus-infected cultures (Fig. 2) remained confluent when maintained in high Ca^{2+} conditions (although growth rate was low)⁴. When these cultures were returned to low Ca^{2+} medium, the confluent cell layer persisted and the cells assumed a basal cell morphology once again. These results support the suggestion that the sarcoma virus (and presumably the active *ras* gene) blocks keratinocytes prior to a step of irreversible commitment to terminal differentiation.

The state of epidermal differentiation is known to determine the response pattern to phorbol ester tumour promoters. Proliferative responses, such as the induction of ornithine decarboxylase (ODC), are characteristic of basal cells^{15,16}, and these responses are rapidly lost as cells begin their maturation programme¹⁵. Phorbol esters markedly accelerate terminal differentiation of more mature basal cells^{16,17}, as indicated by the induction of epidermal transglutaminase and an increase in the percentage of cells with cornified envelopes. Modulation of the normal pattern of phorbol ester responses would be expected if virus infection altered the maturation state of keratinocytes. Virus-infected basal cells incubated in low Ca^{2+} medium are highly sensitive to the induction of ornithine decarboxylase by 12-O-tetradecanoyl phorbol-13-acetate (TPA) (not shown). The response kinetics are similar to those of normal basal cells, although the magnitude is several-fold greater. When normal cells were induced to differentiate by Ca^{2+} , TPA was ineffective in inducing ODC within 6 h after shifting to high Ca^{2+} medium¹⁵ (Fig. 3). However, virus-infected cells retained response to TPA for ODC induction even after several days in high Ca^{2+} medium (Fig. 3). These results are consistent with the suggestion that the virus blocks maturation at the basal cell stage.

Epidermal transglutaminase activity increases during Ca^{2+} -induced differentiation in normal cells¹⁸ and virus infection does not block this increase⁴. We found that in normal cells, transglutaminase activity remained high as the cells completed their

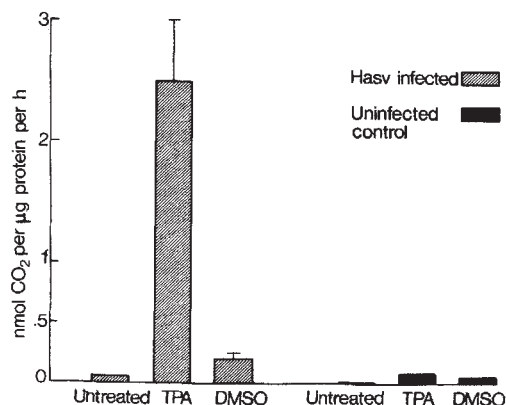


Fig. 3 Ornithine decarboxylase (ODC) response of keratinocytes in 1.2 mM Ca^{2+} medium after exposure to TPA. The viral preparation contained Harvey sarcoma virus with F-MuLV in this experiment, but identical results were obtained with cultures infected with Kirsten sarcoma virus.

Methods. Keratinocytes were plated and exposed to virus as indicated in Fig. 1. Cultures were exposed to virus or polybrene for 72 h in 0.05 mM Ca^{2+} medium and fresh 0.05 mM Ca^{2+} medium for 48 h and then switched to 1.2 mM Ca^{2+} medium for 72 h. Untreated dishes were washed three times with normal saline and frozen on dry ice, while treated cells received fresh 1.2 mM Ca^{2+} medium containing either 0.1% DMSO (Pierce) or 0.1 $\mu\text{g ml}^{-1}$ TPA (Chemical Carcinogenesis, Eden Prairie, Minnesota) and 0.1% DMSO. After a 6-h incubation at 37 °C, cells were washed and frozen as above. Cells from each dish were scraped twice with 120 μl PBS and 50- μl aliquots of the lysates were assayed for ODC and protein as described previously¹⁵.

Table 1 DNA synthesis in epidermal cells after exposure to TPA

Treatment	Control cells (0.5 mM Ca^{2+})	Harvey sarcoma virus-infected cells (0.5 mM Ca^{2+})
	DNA synthesis (c.p.m. per μg DNA)	DNA synthesis (c.p.m. per μg DNA)
0.1% DMSO	511 $\pm$ 59	489 $\pm$ 73
0.0001 $\mu\text{g ml}^{-1}$ TPA	260 $\pm$ 18	616 $\pm$ 27
0.001 $\mu\text{g ml}^{-1}$ TPA	264 $\pm$ 8	665 $\pm$ 52
0.01 $\mu\text{g ml}^{-1}$ TPA	299 $\pm$ 12	953 $\pm$ 175

Virus-infected or control cells were exposed to the test agent while in 0.5 mM Ca^{2+} medium. After 5 days, cultures were exposed to medium containing ^3H -thymidine. Values represent the mean $\pm$ s.e. of four samples for each exposure. DNA synthesis decreases in control cells exposed to TPA and increases in virus-infected cells.

Methods. Epidermal cells were isolated, cultured in 0.05 mM Ca^{2+} medium and infected with virus as described in Fig. 1 legend. Four days after infection, cultures were switched to 0.5 mM Ca^{2+} medium and continuously exposed to TPA or dimethyl sulphoxide (DMSO). After 5 days, the cultures were labelled with 1 $\mu\text{Ci ml}^{-1}$ [^3H]thymidine (6.7 Ci mmol⁻¹, NEN) for 1 h, washed three times with phosphate-buffered saline (PBS) containing 1% unlabelled thymidine and frozen. Quantitation of label incorporated into DNA was performed as described previously¹⁶.

differentiation programme, and this activity was not further influenced by TPA (Fig. 4), although initially TPA and Ca^{2+} acted synergistically to increase transglutaminase activity¹⁷. When virus-infected cells in high Ca^{2+} were exposed to TPA, there was a gradual decrease in transglutaminase activity; in some experiments, activity returned to that seen in low Ca^{2+} . Concurrent with the decrease in transglutaminase activity, TPA also caused a dose-dependent increase in ^3H -thymidine incorporation into DNA of virus-infected but not control cells in high Ca^{2+} (Table 1). These results suggest that maturing keratinocytes expressing an activated *ras* gene respond to phorbol ester tumour promoters by reverting to a less mature basal cell phenotype.

This new information on the biological state of keratinocytes infected with murine sarcoma viruses provides a biological

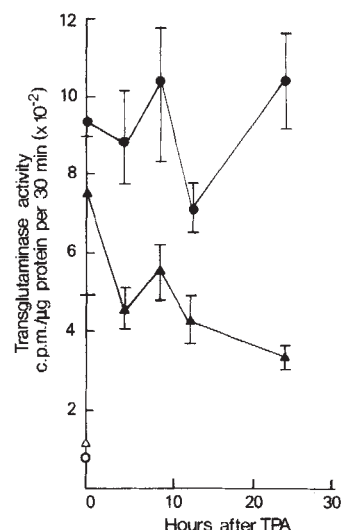


Fig. 4 TPA decreases epidermal transglutaminase in virus-infected cells. ●, Uninfected controls, 1.2 mM Ca^{2+} ; ▲, Kirsten virus-infected, 1.2 mM Ca^{2+} ; ○, uninfected control, 0.05 mM Ca^{2+} ; △, Kirsten virus-infected, 0.05 mM Ca^{2+} . Values represent the mean $\pm$ range of duplicate determinations of duplicate samples. Identical results were obtained with Harvey sarcoma virus-infected cells.

Methods. Keratinocytes were plated and infected with a 1:3 dilution of Kirsten sarcoma virus as in Fig. 1. After 72 h of virus exposure and 24 h of fresh 0.05 mM Ca^{2+} medium, dishes were switched to 1.2 mM Ca^{2+} medium for 48 h. Each group was treated with 0.1 $\mu\text{g ml}^{-1}$ TPA and duplicate samples were washed and frozen on dry ice at 0, 4, 8, 12 and 24 h. Cells were lysed by 5 min incubation at 37 °C with PBS containing 1% Triton X-100 and 10 mM DTT. Transglutaminase was assayed on 50- μl aliquots of the lysate by adding 150 μl of the following freshly prepared mixture: 20 μl of 500 mM sodium borate, pH 9.5, 10 μl of 100 mM CaCl_2 , 10 μl of 10 mM EDTA, 20 μl of casein (20 mg ml⁻¹, Sigma), 10 μl of [2,3- ^3H]putrescine (NEN, 31.3 Ci mmol⁻¹) and 80 μl distilled water. Samples and blanks (containing buffer used for scraping cells instead of cell lysate) were incubated for 30 min at 37 °C and 80- μl aliquots were spotted on Whatman 3MM disks for counting. Protein was measured by the method of Lowry after destroying DTT in 20- μl samples by addition of 250 μl of PBS containing 100 μg chloramine-T and incubation at room temperature for at least 30 min²⁸.

framework to explain how activation of a *ras* gene could yield a phenotype consistent with that hypothesized for initiated cells and required for epidermal tumorigenesis. Although these studies are confined to the effects of v-*ras*, both v-Ki-*ras* and v-Ha-*ras* produce similar effects. Thus, the results are probably relevant to other activated forms of the *ras* gene. We propose that an activated *ras* gene in keratinocytes provides the proliferative signal required to form a tumour, but that the cells are unable to maintain vertical growth above the basement membrane to produce a visible lesion. These cells may be indistinguishable from committed basal cells residing on the basement membrane, which lack proliferative potential¹⁹. Because virus-altered cells fail to complete their differentiation programme, they might be expected to accumulate within the epidermis and expand to a limited extent in the space available¹⁹. Exposure to tumour promoters would selectively reverse the quiescent growth state of those cells with an activated *ras* gene while normal committed basal cells would be unaffected or stimulated to differentiate further¹⁴. Repeated exposure to promoter, such as that required for tumour promotion, would maintain the permissive environment, allowing reactivated cells to grow in the suprabasal regions of the epidermis. Proliferation in the differentiating cell zone is a distinguishing feature of epidermal papillomas⁵.

In many mouse strains, at least two classes of skin papillomas develop in protocols involving initiation and promotion²⁰. Promoter-dependent tumours require repeated exposure to promoter for both expression and maintenance and regress on

withdrawal of the promoting stimulus²⁰. Autonomous papillomas, once formed, do not require promoter exposure for maintenance and are more likely to progress to carcinomas^{20,21}. The biological changes we have defined for keratinocytes expressing an activated *ras* gene are most consistent with *ras* activation being involved in the formation of promoter-dependent tumours. In contrast, the characteristics of chemically altered cells, selected by virtue of their resistance to differentiation, are most consistent with changes in autonomous papillomas. Whether the latter cell type requires more than one genetic change (of which *ras* activation could be a component and thus explain the isolation of an activated *ras* from 60% of papillomas) or an alteration in a different genetic element remains to be determined.

Both chemically altered and virus-altered cells fail to produce

epidermal carcinomas in nude mice (ref. 22 and our unpublished data). Thus *ras* activation alone is not sufficient to complete the cancer process. We have shown previously that at least two genetic events are required to produce carcinomas in mouse skin²³. Other studies have suggested that several viral oncogenes must work in concert to transform primary cultures of murine cells²⁴⁻²⁶. Weissman and Aaronson have shown that murine sarcoma viruses can elicit the transformed phenotype in aneuploid mouse epidermal cells²⁷. Thus, the combination of an activated *ras* gene and aneuploidy may satisfy additional requirements for transformation.

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- Kulesz-Martin, M., Koehler, B., Hennings, H. & Yuspa, S. H. *Carcinogenesis* 1, 995-1006 (1980).
- Yuspa, S. H. & Morgan, D. L. *Nature* 293, 72-74 (1981).
- Yuspa, S. H. in *Cellular Interactions by Environmental Tumor Promoters* (eds Fujiki, H., Hecker, E., Moore, R. E., Sugimura, T. & Weinstein, I. B.) 315-326 (Japan Scientific Societies Press, Tokyo).
- Yuspa, S. H., Vass, W. & Scolnick, E. *Cancer Res.* 43, 6021-6030 (1983).
- Yuspa, S. H., Hennings, H. & Lichti, U. *J. supramolec. Struct. Cell Biochem.* 17, 245-247 (1981).
- Balmain, A., Ramsden, M., Bowden, G. T. & Smith, J. *Nature* 307, 658-660 (1984).
- Hennings, H. *et al. Cell* 19, 245-254 (1980).
- Kulesz-Martin, M., Kilkenny, A. E., Holbrook, K. A., Digernes, V. & Yuspa, S. H. *Carcinogenesis* 4, 1367-1377 (1983).
- Balmain, A. & Pragnell, I. B. *Nature* 303, 72-74 (1983).
- Stanley, J. R., Hawley-Nelson, P., Yuspa, S. H., Shevach, E. M. & Katz, S. I. *Cell* 24, 897-903 (1981).
- Stanley, J. R., Woodley, D. T. & Katz, S. I. *J. invest. Derm.* 82, 108-111 (1984).
- Stanley, J. R., Koulou, C. & Thivolet, C. *J. clin. Invest.* 74, 313-320 (1984).
- Stanley, J. R. & Yuspa, S. H. *J. Cell Biol.* 96, 1809-1814 (1983).
- Hennings, H. & Holbrook, K. in *Ions, Cell Proliferation and Cancer* (eds Boynton, A. L., McKeehan, W. L. & Whitfield, J. F.) 499-516 (Academic Press, New York, 1982).
- Lichti, U., Patterson, E., Hennings, H. & Yuspa, S. H. *J. cell. Physiol.* 107, 261-270 (1981).
- Yuspa, S. H., Ben, T., Hennings, H. & Lichti, U. *Cancer Res.* 42, 2344-2349 (1982).
- Yuspa, S. H., Ben, T. & Hennings, H. *Carcinogenesis* 4, 1413-1418 (1983).
- Hennings, H., Steinert, P. & Buxman, M. M. *Biochem. biophys. Res. Commun.* 102, 739-745 (1981).
- Potten, C. S. *Int. Rev. Cytol.* 69, 271-318 (1981).
- Burns, F. J., Vanderlaan, M., Sivak, A. & Albert, R. E. *Cancer Res.* 36, 1422-1427 (1976).
- Scribner, J. D., Scribner, N. K., McKnight, B. & Mottet, N. K. *Cancer Res.* 43, 2034-2041 (1983).
- Kilkenny, A. E., Morgan, D., Spangler, E. F. & Yuspa, S. H. *Cancer Res.* (in the press).
- Hennings, H. *et al. Nature* 304, 67-69 (1983).
- Newbold, R. F. & Overell, R. W. *Nature* 304, 648-651 (1983).
- Land, H., Parada, L. F. & Weinberg, R. A. *Nature* 304, 596-602 (1983).
- Ruley, E. H. *Nature* 304, 602-606 (1983).
- Weissman, B. E. & Aaronson, S. A. *Cell* 32, 599-606 (1983).
- Higuchi, M. & Yoshida, F. *Analyt. Biochem.* 77, 542-547 (1977).
- Toftgard, R., Roop, D. R. & Yuspa, S. H. *Carcinogenesis* (in the press).

Cigarette smoke induces DNA single-strand breaks in human cells

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Epidemiological evidence suggests that smoking is a major cause of human lung cancer^{1,2}. However, the mechanism by which cigarette smoke induces the cancer remains obscure, although in tobacco carcinogenesis, promotion and/or co-carcinogenesis may have crucial roles. The epidemiological data show that if an individual stops smoking, the risk of his contracting lung cancer increases no further. Moreover, laboratory experiments show that cigarette smoke condensate (CSC) exhibits co-carcinogenic and promoting activities in tumour production and malignant transformation³⁻⁵. Clastogenic action is thought to be intimately involved in tumour promotion⁶⁻⁸, and it is therefore interesting that visible chromosome changes such as chromosome aberrations and sister chromatid exchanges are known to be caused by cigarette smoke⁹⁻¹¹. However, there has been no previous direct demonstration that cigarette smoke can cause single-strand breaks (SSB) in DNA. Here we report that cigarette smoke induces considerable numbers of DNA SSB in cultured human cells, and that such strand breaks may be ascribed to active oxygen generated from cigarette smoke.

Alkali-labile sites and SSB in DNA were analysed by an alkaline elution technique¹². Smoke from a commercial filter-tipped cigarette was passed through 6 ml of phosphate-buffered saline (PBS), pH 7.4, for 5 min and used immediately as 'smoke-PBS' (ref. 13). Human lung carcinoma (A549) cells prelabelled with ¹⁴C-thymidine (50 nCi per nmol per Petri dish) were treated with smoke-PBS at 20 °C for 1 h.

Figure 1a shows the alkaline elution profile of human cell DNA after treatment with 1 ml of smoke-PBS. The cells showed ~50% plating efficiency after treatment with this volume of

Table 1 Effect of scavengers on DNA strand breaks caused by cigarette smoke or by H₂O₂ in a cell system

Treatment	SSB per 10 ¹⁰ daltons	%SSB relative to standard
Expt 1		
Smoke-PBS	55.1 ± 3.2	100
Smoke-PBS + SOD (100 µg)	35.4 ± 3.1	64
Expt 2		
Smoke-PBS	21.1 ± 0.7	100
Smoke-PBS + catalase (2 µg)	3.4 ± 0	16
Smoke-PBS + inactivated catalase (2 µg)	22.1 ± 4.2	104
Expt 3		
H ₂ O ₂ (32 µM)	9.5 ± 0.8	100
H ₂ O ₂ (32 µM) + catalase (2 µg)	1.6 ± 0.2	17
H ₂ O ₂ (32 µM) + inactivated catalase (2 µg)	8.3 ± 2.2	87

The reaction mixture contained 3 × 10⁵ cells and 1.0 ml of smoke-PBS. The specific activities of catalase (Sigma) and SOD (Sigma) were 20,000 U mg⁻¹ and 3,000 U mg⁻¹, respectively. Inactivated catalase was prepared as follows. Catalase (2 mg) was incubated in 1 ml of 0.1 M NaOH at 70 °C for 30 min and neutralized with 0.1 M HCl. Each experiment was performed in duplicate. Other conditions were the same as in Fig. 1.

smoke-PBS and, according to dye exclusion tests, 100% of the cells excluded trypan blue immediately after smoke-PBS treatment. The number of SSB obtained using different amounts of smoke-PBS (Fig. 1b) was calculated by external standardization (4.05 SSB per 10¹⁰ daltons of DNA were produced in cells irradiated at 4 °C with 150 rad of γ rays of ⁶⁰Co at 37.5 rad min⁻¹). The number of SSB varied almost fivefold between experiments, probably because of the fluctuating conditions for trapping smoke and a subtle difference in the quality of individual cigarettes. However, when the same smoke-PBS was used, consistent results were obtained.

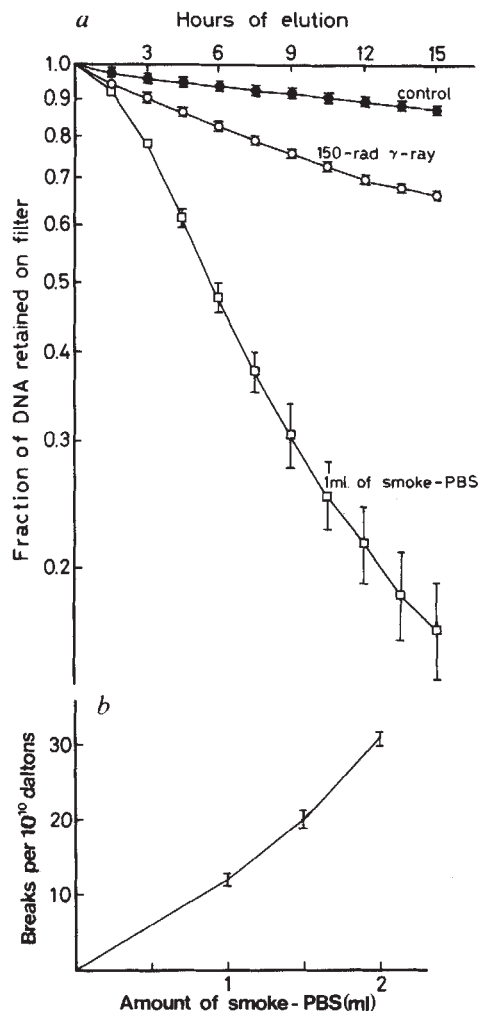


Fig. 1 *a*, Alkaline elution profiles of DNA from cells treated with 1 ml of PBS solution of cigarette smoke. *b*, Effects of different amounts of smoke-PBS on DNA strand breaks.

Methods. A549 cells (5×10^5 cells per dish) labelled with ^{14}C -thymidine were preincubated at 20°C with 40 mM HEPES buffer (pH 7.2) containing heat-inactivated fetal calf serum at a final concentration of 7%. After 1 h incubation with (□) or without (●) smoke-PBS at 20°C , the cells were washed twice with ice-cold PBS and collected with a rubber stripper in PBS on ice. (As a standard for DNA strand breaks, cells were irradiated with 150 rad γ -rays from ^{60}Co and collected in the same way (○).) The cells were lysed on a polycarbonate filter (pore size $2.0 \mu\text{m}$) with proteinase K (0.5 mg ml^{-1}) in the presence of 2% SDS, washed with 20 mM EDTA (pH 9.6) and eluted with 50 mM tetrapropylammonium hydroxide buffer containing 20 mM EDTA (pH 11.9) at a rate of 0.05 ml min^{-1} . Fractions were collected directly in scintillation vials at 90-min intervals. Radioactivity remaining on the filters was recovered by heating the filter at 70°C for 1 h in 0.5 ml of 1 M HCl followed by the addition of 1 M NaOH. All experiments were performed in duplicate and the bar shows two original points in each experiment.

It was important to identify the component in cigarette smoke that is responsible for DNA SSB. Previously, we found that large amounts of active oxygen such as H_2O_2 and O_2^- were generated from cigarette smoke after trapping the smoke in PBS ($\sim 0.5 \mu\text{g ml}^{-1}$ of H_2O_2 was regularly detected in smoke-PBS)¹³. We also showed that SSB in DNA were produced by active oxygen radicals generated from active metabolites of carcinogenic aromatic amines¹². These findings suggested that the active oxygen generated from cigarette smoke might be responsible for the observed SSB in DNA. To verify this, we checked the effect of catalase and superoxide dismutase (SOD) on the number of SSB produced, and found that both enzymes inhibited SSB, the inhibition with catalase being greater than with SOD (Table 1). Although the absolute number of SSB varied in several

Fig. 2 Effect of different amounts of smoke-PBS on DNA strand breaks in a cell-free system. A549 cells (4×10^5 cells per dish) labelled with ^{14}C -thymidine were collected by trypsin treatment, put on polycarbonate filters and lysed with proteinase K (0.5 mg ml^{-1}) in the presence of 2% SDS. The filters were washed twice with 20 mM EDTA (pH 9.6), then 5 ml PBS, 20 mM EDTA (pH 7.4) containing the indicated amount of smoke-PBS was added to the cell lysate on the filter with the outlet of the filter closed. The reaction mixture was kept in the dark at room temperature for 1 h, and the filters were then washed twice with 3 ml 20 mM EDTA (pH 9.8) and eluted with tetrapropylammonium hydroxide buffer (pH 11.9) containing 20 mM EDTA at a rate of 0.05 ml min^{-1} . The subsequent treatment and calculation of SSB were the same as for the cell system (see Fig. 1 legend).

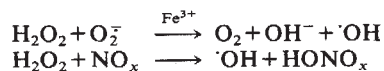
Table 2 Effect of scavengers on DNA strand breaks caused by cigarette smoke in a cell-free system

Treatment	% SSB relative to standard
Standard	100
+SOD (10 μg)	11
+catalase (2 μg)	8
+inactivated catalase (2 μg)	100
+sodium benzoate (50 mM)	18
+DETAPAC (1 mM)	44

The standard system includes 5 ml of PBS (pH 7.4) containing 20 mM EDTA and $50 \mu\text{l}$ of smoke-PBS, and was incubated at room temperature for 1 h. Each value was obtained from four independent experiments performed in duplicate. The average error was about 10% in each duplicated experiment. The SSB value of the standard was 6.2 ± 1.6 (mean $\pm$ s.d.) SSB per 10^{10} daltons.

independent experiments, the inhibition rates of the two enzymes showed a similar range (catalase, 85–95%; SOD, 30–45%). Inactivated catalase showed no effect in these inhibition experiments. Comparison of the activity of catalase and inactivated catalase in parallel, using authentic H_2O_2 , showed clearly that the inhibitory effect of catalase was a result of its enzyme activity of quenching H_2O_2 .

To exclude completely the contribution of DNA repair in SSB formation and to elucidate the mechanism of DNA scission more precisely, we looked for SSB in cell-free *in vitro* systems. Strand breaks were detected in protein-free DNA of high relative molecular mass which had been treated with smoke-PBS on polycarbonate filters, the number of SSB increasing dose dependently (Fig. 2). Table 2 shows the effect of several scavengers in a cell-free system. Catalase and SOD decreased the number of SSB, as did sodium benzoate, a hydroxyl radical ($\cdot\text{OH}$) scavenger, while 1 mM diethylenetriamine- N,N,N',N'' -pentaacetic acid (DETAPAC), a potent Fe^{3+} chelator, inhibited SSB in the presence of 20 mM EDTA. In cigarette smoke, $\cdot\text{OH}$ could be formed through the Fenton-type Haber-Weiss reactions and/or the reaction of H_2O_2 with nitrogen oxides^{13,14}.



Accordingly, it is probable that the SSB observed were caused by $\cdot\text{OH}$ that had been formed by these reactions, which in their turn had been induced by cigarette smoke. From these results, we conclude that cigarette smoke has clastogenic activity on cellular DNA to produce SSB as detected by alkaline elution, and that such activity can be explained mainly by the action of active oxygen generated from cigarette smoke.

Although mutagenicity induced by CSC has been found in bacterial and mammalian cells⁹, this does not necessarily mean that the mutagenic compounds are directly involved in tobacco carcinogenesis¹⁵. On the other hand, the frequency of benzo(a)pyrene-induced transformation of Syrian hamster embryo cells is enhanced by CSC as efficiently as by the tumour promoter phorbol myristate acetate^{4,5}. These facts and the recent findings that the clastogenic action of active oxygen is involved in the promoting process suggest an important role for DNA SSB induced by cigarette smoke⁶⁻⁸. The active oxygen is generated mostly from polyphenols, such as catechol, its methyl derivatives and hydroquinone, which occur in abundance in the smoke¹³. This is consistent with the previous finding that the co-carcinogenic fraction in CSC contains catechol and hydroquinone and that catechol shows potent co-carcinogenic action

in benzo(a)pyrene carcinogenesis¹⁶.

A good correlation has been found between free radical formation, generation of active oxygen and carcinogenicity by aromatic amine derivatives and other carcinogens¹⁷⁻²³. This may imply a causal significance of SSB in carcinogenesis, because the SSB in DNA were generated by active oxygen from active metabolites of naphthylamines¹². In the present experiments, one cigarette produced ~10⁴ SSB per cell. Although in the body, most SSB caused by cigarette smoke are expected to be efficiently repaired, some would remain unrepaired and the accumulation of such SSB over a long period may have serious consequences, especially for heavy smokers.

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1. Doll, R. & Peto, R. *Br. med. J.* **2**, 1525-1536 (1976).
2. Doll, R. *Cancer Res.* **38**, 3573-3583 (1978).
3. Hecht, S. S., Carmella, S., Mori, H. & Hoffman, D. *J. natn. Cancer Inst.* **66**, 163-169 (1981).
4. Rivedal, E. & Sanner, T. *Cancer Lett.* **10**, 193-198 (1980).
5. Rivedal, E., Hemstad, J. & Sanner, T. *Mechanisms of Toxicity and Hazard Evaluation* (eds Homstedt, B., Lauwerys, R., Mercier, M. & Roberfroid, M.) 259-263 (Elsevier, Amsterdam, 1980).
6. Emerit, I. & Cerutti, P. A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7509-7513 (1980).
7. Birnboim, H. C. *Science* **215**, 1247-1249 (1982).
8. Copeland, E. S. *Cancer Res.* **43**, 5631-5637 (1983).
9. DeMarini, D. M. *Mutat. Res.* **114**, 59-89 (1983).
10. Hopkin, J. M. & Evans, H. J. *Nature* **279**, 241-242 (1979).
11. Benedict, W. F. *et al. Mutat. Res.* **136**, 73-80 (1984).
12. Kaneko, M., Nakayama, T., Kodama, M. & Nagata, C. *Gann* **75**, 349-354 (1984).
13. Nakayama, T., Kodama, M. & Nagata, C. *Gann* **75**, 95-98 (1984).
14. Pryor, W. A. *Ann. N. Y. Acad. Sci.* **393**, 1-22 (1982).
15. Wynder, E. L. & Hoffman, D. in *Tobacco and Tobacco Smoke, Studies in Experimental Carcinogenesis*, 225-231 (Academic, New York, 1967).
16. Van Duuren, B. L. & Goldschmidt, B. M. *J. natn. Cancer Inst.* **56**, 1237-1242 (1976).
17. Hozumi, M. *Gann* **60**, 83-90 (1969).
18. Kimura, T., Kodama, M. & Nagata, C. *Carcinogenesis* **3**, 1393-1396 (1982).
19. Nakayama, T., Kimura, T., Kodama, M. & Nagata, C. *Carcinogenesis* **4**, 765-769 (1983).
20. Stier, A., Clauss, R., Lücke, A. & Reitz, I. *Xenobiotica* **10**, 661-673 (1980).
21. Floyd, R. A., Soong, L. M., Stuart, M. A. & Reigh, D. L. *Photochem. Photobiol.* **28**, 857-862 (1978).
22. Nagata, C., Kodama, M., Ioki, Y. & Kimura, T. in *Free Radicals and Cancer* (ed. Floyd, R. A.) 1-62 (Dekker, New York, 1982).
23. Lesko, S. A., Lorentzen, R. J. & Ts'o, P. O. P. in *Polycyclic Hydrocarbons and Cancer Vol. 1* (eds Gelboin, H. V. & Ts'o, P. O. P.) 261-269 (Academic, New York, 1978).

Characterization of cDNA and genomic clones encoding the precursor to rat hypothalamic growth hormone-releasing factor

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Growth hormone-releasing factor (GHRF) is a hypothalamic peptide which positively regulates the synthesis and secretion of growth hormone in the anterior pituitary. The amino-acid sequence of a 43-residue GHRF peptide isolated from rat hypothalamus was recently determined¹. Immunocytochemical techniques have been used to localize GHRF-containing cell bodies and nerve fibres largely to the medial-basal region of the rat hypothalamus². The rat has also been used extensively as an animal model to study the effects of GHRF on growth hormone synthesis^{3,4} and secretion^{5,6} and on somatic growth⁷. To pursue questions concerning the biosynthesis of GHRF, the expression of the *ghrf* gene, and its regulation in the hypothalamus by neural and hormonal influences, we have now isolated and characterized both complementary DNA and genomic clones encoding rat hypothalamic GHRF. The rat *ghrf* gene spans nearly 10 kilobases (kb) of rat genomic DNA, contains 5 exons and encodes a 104-amino-acid precursor to the rat GHRF peptide. Comparison with previously characterized human *ghrf* cDNA^{8,9} and genomic¹⁰ clones has allowed patterns of conservation of amino-acid and nucleotide sequences between the human and rat GHRFs to be determined.

A 100,000-member cDNA library was constructed in the bacterial expression vector pUC8 (ref. 11) using as template poly(A)⁺ RNA isolated from rat medial-basal hypothalamus, which was shown previously by immunohistochemical techniques to contain a concentration of GHRF-reactive cell bodies^{2,12}. This library was screened by hybridization using the

insert from a human *ghrf* cDNA clone as probe⁹, and two positive colonies were identified and isolated. Figure 1a shows the DNA sequence of the insert from one of these clones, prghrf-2. This cDNA includes a sequence encoding the complete 43-amino-acid rat GHRF peptide predicted from the amino-acid sequence data of Vale and co-workers¹. The structure of the entire rat *ghrf* cDNA insert is shown schematically in Fig. 1b. The GHRF peptide sequence is followed by a single arginine residue which presumably serves as a signal for proteolytic processing of GHRF from the precursor protein¹³. This arginine is not preceded by a glycine residue, which is believed to serve as the amide donor in processed peptides that are C-terminally amidated¹⁴. This agrees with the published amino-acid sequence suggesting a free C-terminus¹, and makes rat GHRF different from human, bovine, ovine, porcine and caprine GHRFs, all of which are C-terminally amidated¹⁵. In cDNA clone prghrf-2, the GHRF peptide sequence is followed by a 30-amino-acid peptide of unknown function, a 105-nucleotide 3'-non-translated sequence and a poly(A) tract (Fig. 1b). Comparison with previously characterized human *ghrf* cDNA clones^{8,9} suggests that this rat *ghrf* cDNA clone lacks the N-terminal portion of the precursor, including the presumed signal peptide sequence and the 5'-non-translated sequences.

The rat *ghrf* cDNA clone was used to screen a rat genomic library constructed in the λ vector Charon 4A (ref. 16). Figure 2a shows the structures of two overlapping clones isolated from this library (rghrf λ 101 and rghrf λ 103) which predict the entire structure of the rat *ghrf* gene. Hybridization identifies 5 exons which span more than 9 kb of rat genomic DNA, and reveals a structure very similar to that previously determined for the human *ghrf* gene¹⁰. Southern blot analysis of rat genomic DNA indicates that the rat *ghrf* gene is probably single-copy (data not shown). To define the structure of this gene more precisely, the DNA sequences of all exons, intron/exon boundaries and 5'- and 3'-flanking regions were determined by the strategy indicated (Fig. 2b). Figure 3 gives the DNA sequence of the rat *ghrf* gene. All four introns begin and end, respectively, with the consensus sequences GT and AG¹⁷. Consensus TATA and CAT sequences, found in the 5'-flanking region of many eukaryotic genes^{17,18}, are located 31 and 76 nucleotides, respectively, 5' of the proposed messenger RNA cap site. The location of the proposed mRNA cap site is based on its distance from the TATA

Fig. 1 Sequence and structure of a cDNA clone encoding rat hypothalamic growth hormone-releasing factor. *a*, DNA sequence of the insert from rat *ghrf* cDNA clone prghrf-2. The amino-acid sequence predicted from the open reading frame within the insert is shown beneath the DNA sequence. *EcoRI* and *Sall* indicate the synthetic linkers ligated to the 5' and 3' ends of the cDNA insert, respectively. The sequence of the 43-amino-acid rat GHRF peptide is underlined. *b*, Diagram of the structure of rat GHRF cDNA clone prghrf-2. The *ghrf*-coding region, the 30-amino-acid C-terminal peptide, the 3'-non-translated region and the poly(A) tract are indicated. The positions of the *Escherichia coli* β -galactosidase initiation codon within the vector and the natural stop codon with the insert are also indicated. The restriction map indicates sites for common 4- and 6-bp cutters within the insert, and the arrows indicate the strategy used for DNA sequence analysis of the insert. **Methods.** Poly(A)⁺ RNA was isolated using standard techniques from medial-basal hypothalamic fragments obtained from 150-g Zivic-Miller male rats that had been adrenalectomized for other purposes 3–4 days before they were killed. (These fragments were kindly provided by Dr Michael Brownstein, NIH.) This RNA was used as a template for oligo(dT)-primed first-strand cDNA synthesis with avian myeloblastosis virus reverse transcriptase. Second-strand cDNA synthesis was performed using the large fragment of DNA polymerase I, and *Sall* and *EcoRI* linkers were added sequentially to the 3' and 5' ends of the double-stranded cDNA, respectively. Approximately 100 ng of cDNA were ligated into the bacterial expression vector pUC8 that had previously been digested with *EcoRI* and *Sall* and the ligated mixture was used to transform CaCl₂-competent *E. coli* strain DH-1. Bacteria were plated at a density of 1,000 colonies per 82-mm plate onto L-broth agar with ampicillin, replica plated to nitrocellulose filters, and the filters screened by hybridization to the 350-bp *EcoRI*/*BamHI* insert from human *ghrf* cDNA clone phghrf-54 (ref. 9), which had been labelled by nick-translation using [α -³²P]dCTP. Positive colonies from the high-density screen were re-screened at lower density until pure. Two positive colonies were isolated from 100,000 recombinants screened. Because initial analysis indicated that both clones contained similarly sized inserts, only one was analysed in detail. Subsequent analysis indicated that the second cDNA clone contained a single additional G residue at the 5' end of the insert. All cloning and screening procedures have been described in detail elsewhere²³. DNA sequence analysis was by the chemical method of Maxam and Gilbert²⁴, using fragments that had been labelled using bacterial alkaline phosphatase, T4 polynucleotide kinase and [γ -³²P]ATP. Enzymes were from either Bethesda Research Laboratories or New England Biolabs, and labelled nucleotides were from NEN.

a

5'-ATG ACC ATG ATT ACG AAT TCC
Eco RI

CAT GCA GAC GCC ATC TTC ACC AGC AGC TAC CGG AGA ATC CTG GGC	45
His Ala Asp Ala Ile Phe Thr Ser Ser Tyr Arg Arg Ile Leu Gly	
CAA TTA TAT GCC CGC AAA CTG CTG CAC GAA ATC ATG AAC AGG CAG	90
Gln Leu Tyr Ala Arg Lys Leu Leu His Glu Ile Met Asn Arg Gln	
CAA GGG GAG AGG AAC CAG GAA CAA AGA TCC AGG TTC AAC CGC CAT	135
Gln Gly Glu Arg Asn Gln Glu Gln Arg Ser Arg Phe Asn Arg His	
TTG GAC AGA GTG TGG GCA GAG GAC AAG CAG ATG GCC CTG GAG AGC	180
Leu Asp Arg Val Trp Ala Glu Asp Lys Gln Met Ala Leu Gln Ser	
ATC TTG CAG GGA TTC CCA AGG ATG AAG CTT TCA GCG GAG GCT TGA	225
Ile Leu Gln Gly Phe Pro Arg Met Lys Leu Ser Arg Ala Glu Ala	
GCC CTC GGC CCC CAA ACA TAG CTG GAC CCT GTT ACT TCT ACT TCA	270
GTT CTG ATC TTC TCC TTC CTC TGT GAA TAC AAT AAA GAC CCA TCT	315
CAT CTG CAC TTC A(32) G GTC GAC -3	
Sall	

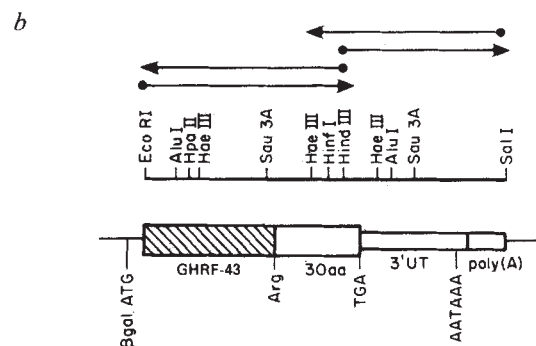
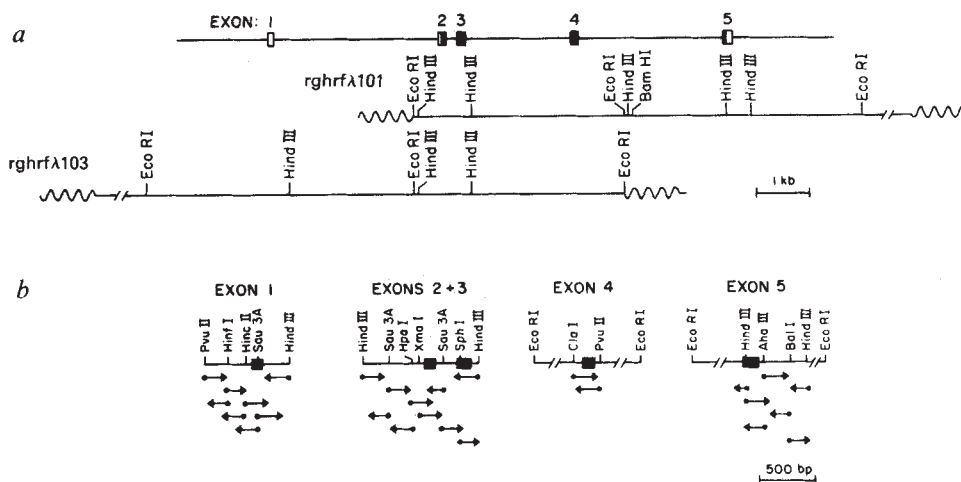


Fig. 2 Structure of rat growth hormone-releasing factor genomic clones and subclones. *a*, The top line indicates the deduced structure of the entire rat *ghrf* gene. Open boxes indicate non-coding exon regions and closed boxes indicate coding exon regions. Below this are indicated the structures of λ phage genomic clones rghrf λ 101 and rghrf λ 103. *EcoRI*, *BamHI* and *HindIII* restriction enzyme sites used to establish overlap between the clones are indicated. The wavy lines indicate the λ vector DNA, slashes indicate a break in the scale. *b*, Regions of the above λ phage clones which were subcloned into plasmid vectors for further analysis are indicated, as is the strategy used to determine the DNA sequence of the rat *ghrf* gene. Boxed regions are exons as indicated and selected restriction enzyme recognition sites used in the sequence analysis are shown. Slashes represent a break in the scale. Dots represent sites that were labelled for DNA sequencing and the arrows indicate the direction and extent of derived sequence information.



Methods. Approximately 400,000 plaques from a rat genomic library consisting of partial *EcoRI*-digested rat DNA in the λ phage vector Charon 4A¹⁶ were screened on nitrocellulose filters by hybridization to the 350-bp *EcoRI*/*Sall* insert from rat *ghrf* cDNA clone prghrf-2 which had been labelled by nick-translation using [α -³²P]dCTP. Two types of clones, represented by those shown in *a*, were isolated. Exon regions were located by restriction mapping/DNA blotting techniques using appropriate probes. The rat cDNA clone was used as probe to identify exons 3, 4 and 5. Human *ghrf* cDNA clone phghrf-54 (ref. 9) was used to locate rat exon 2, and genomic probes isolated from the human *ghrf* gene¹⁰ were used to localize rat exon 1. Regions from the genomic clones indicated in *a* were subcloned into plasmid pBR322 for DNA sequence analysis, which was by the chemical method of Maxam and Gilbert²⁴. Details of all cloning and mapping methods have been described previously²³. Materials were from the sources indicated in Fig. 1 legend.

box^{17,18}, the fact that transcription generally begins at an A residue¹⁹, and its homology to the cap site of the human *ghrf* gene, which has been determined by RNA mapping techniques¹⁰. The sequence AATAAA, thought to be important in polyadenylation²⁰, is located 21 nucleotides 5' of the poly(A) addition site.

A combined analysis of the rat *ghrf* cDNA and genomic clones allows the entire amino-acid sequence of the rat GHRF precursor protein to be deduced. This sequence is compared with that of the human GHRF precursor protein in Fig. 4a. The GHRF peptides themselves are closely homologous, but differ substantially at their C-terminus (Fig. 4a). Because most of the

GTATAATCAATGGCAGGACATTCTGATAATGGATGACAGGGAGACAGTGAACAAT
 -400
 TTTCCCACTAAGAGGGAACCAATTTGGTGGTAATTTGTCTATTCATAGTGAA
 -350
 AGAAAGTTACGTGTGAACAAACAGAAATATAGGGAACCTGGAAAGCATAGTTTATGGG
 -300
 AGAGTGAGCAAGCCATGACATTGTGGTAAATAAATACTAGACTCTTACTGTTATTCA
 -250
 CCATTTTCTCAGAATAATCATTCAACATTATTTCTGTTACCCCCACAAAGACGAAGG
 -200
 AGAAGTTCGGTATGGTCTAAATAGTGGGGGCACAGAAACCATTTCCTCCATTAGTTT
 -150
 GCGTGATGCTAAATGGGCATTGGTGTGACTTCTGCGCAATGACTCTCTGACCGTGA
 -100
 CAACACTTAGGGAATGAAGGTATAAATGTGAGTTACGGCAGTGGCTGGAGCAGATGCC
 -50
 AGCCTGAGGTGAGTGGGACCTGAGCAGCATCTGATCGGAGAGGGATACCTGTCACCTCA
 Exon 1
 GAGGTGAGGGGGG.....Intron A: 3.2 kb.....CTTCCTTCAGTCCCGC
 100
 CAGGAGTGAAGGATGCCACTCTGGGTGTTCTTTGTGCTCTCACCCTCACCAGTGGCTCC
 MetProLeuTrpValPhePheValLeuLeuThrLeuThrSerGlySer
 150
 CACTGCTCACTGCCCCCTCACTCCCTTCAGTGAACGAGCA.....Intron B
 HisCysSerLeuProProSerProPheAr
 Exon 3
 230 bp.....CTTTCACAGGGTGGCGGGCATGACAGCCATCTTACCAGCAGC
 gValArgArgHisAlaAspAlaIlePheThrSerSer
 250
 TACCGGAGATCCTGGGCAATTATATGCCCGCAACTGCTGCAGGAATCATGAACAGG
 TyrArgArgIleLeuGlyGlnLeuTyrAlaArgLysLeuLeuHisGluIleMetAsnArg
 Exon 4
 CAGCAAGGTAAGCAGAC.....Intron C: 2.0 kb.....GAACCCATAGG
 GlnGlnGln
 300
 GAGAGGAACAGGAACAAAGATCCAGGTCAACCGCCATTGGACAGAGTGTGGGACAG
 GlnArgAsnGlnGluGlnArgSerArgPheAsnArgHisLeuAspArgValTrpAlaGlu
 350
 GACAAGCAGATGGCCCTGGAGAGCATCTTGTAACCTTCG.....Intron D
 AspLysGlnMetAlaLeuGluSerIleLeu
 Exon 5
 2.8 kb.....CTTTCGACGAGGATTCACCAAGGATGAAGCTTTCAGCGGAGGCTT
 GlnGlyPheProArgMetLysLeuSerAlaGluAla
 400
 GAGCCCTCGGCCCAACATAGCTGGACCTGTTACTTCTACTTCAGTTCGATCTTCT
 450
 CCTTCCTCTGTGAATAAAGACCCATCTCATCTGCACTTCAGTTCCTTCATTGTCT
 500
 AATCTGATTGTTTTTAAACGTGGTATGTTTACCTCTCCCAAGTAGCATGACAAGT
 ATAATGATGACAGCAGCAGAGCGGAGAGATGGCTCAGTTGGTAAGCTTTTTCGTGAC
 AAGCATGAGGAGCTCAGACCTTACGACCCACATAAAGCCAGGCTCAGGAGTGATCG

Fig. 3 DNA sequence of the rat growth hormone-releasing factor gene. The DNA sequences of all exons, intron/exon boundaries and 5'- and 3'-flanking regions are shown. Numbering is from the proposed mRNA 5' cap site and the introns are not numbered. Arrows indicate the beginning of exons, or the poly(A) addition site (last arrow). The larger letters represent exons of the gene; exons were deduced either by identity with the rat *ghrf* cDNA or by homology with the exons of the human *ghrf* gene. The first and last 10 nucleotides of each intron are shown together with an approximate length of each intron. The amino acids indicated in italic print are those of the 43-amino-acid GHRF peptide. The boxed nucleotides indicate consensus sequences as described in the text; they are the CAT box, TATA box and AATAAA sequence, in that order.

biological activity of GHRF is localized to the N-terminal two-thirds of the peptide^{21,22}, changes in the C-terminal portion of the peptide can apparently be tolerated without loss of function. Proteolytic processing of GHRF from its precursor protein is expected to yield a C-terminal peptide of unknown function. Comparison of the human and rat precursor sequences (Fig. 4a) shows that the N-terminal half of this peptide is well conserved, whereas the C-terminal half diverges completely between rat and human. Analysis of the rat *ghrf* gene sequence (Fig. 3) reveals that this point of divergence corresponds precisely to a point where the precursor coding sequence is interrupted by intron D. Although the DNA sequence in this region is conserved between the human and rat *ghrf* genes, an apparent mutational event has resulted in a change in the 5' splice donor site used in the human versus the rat gene, shifting the translational reading frame (data not shown). Comparison of the human and rat *ghrf* gene DNA sequences indicates that although the two genes are homologous throughout, a particularly high degree of homology is found in their 5'-flanking regions (Fig. 4b). The sequence from -23 to -109 is 86% identical, compared with 76% identity between the *ghrf*-coding regions, 68% identity

a

Human: Met-Pro-Leu-Trp-Val-Phe-Phe-Phe-Val-Ile-Leu-Thr-Leu-Ser-Asn-
 Rat: Met-Pro-Leu-Trp-Val-Phe-Phe-Val-Ile-Leu-Thr-Leu-Thr-Ser-
 Ser-Ser-His-Cys-Ser-Pro-Pro-Pro-Pro-Leu-Thr-Leu-Arg-Met-Arg-
 Gly-Ser-His-Cys-Ser-Leu-Pro-Pro-Ser-Pro-Pro-Phe-Arg-Val-Arg-
 Arg-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-
 Arg-His-Ala-Asp-Ala-Ile-Phe-Thr-Ser-Ser-Tyr-Arg-Arg-Ile-Leu-
 Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-
 Gly-Gln-Leu-Tyr-Ala-Arg-Lys-Leu-Leu-His-Glu-Ile-Met-Asn-Arg-
 Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-
 Gln-Gln-Gly-Glu-Arg-Asn-Gln-Glu-Gln-Arg-Ser-Arg-Phe-Asn-
 Gly-Arg-Gln-Val-Asp-Ser-Met-Trp-Ala-Glu-Gln-Lys-Gln-Met-Glu-
 Arg-His-Leu-Asp-Arg-Val-Trp-Ala-Glu-Asp-Lys-Gln-Met-Ala-
 Leu-Glu-Ser-Ile-Leu-Val-Ala-Leu-Leu-Gln-Lys-His-Ser-Arg-Asn-
 Leu-Glu-Ser-Ile-Leu-Gln-Gly-Phe-Pro-Arg-Met-Lys-Leu-Ser-Ala-
 Ser-Gln-Gly-Stop (108 aa)
 Glu-Ala-Stop (104 aa)

b

Human: CTTTACCAACAGGGTTGTGAGTGTGATGATGCTAAAAACAGTCC
 Rat: CAGAAACCACTTTTCCCATTTAGTTTTCGTTGATGCTAAAAACGCGC
 -120 -100
 -80 -60
 -TTTGGTTGACTTGTGGGTAATTGATCTCTGACGCTGACAACGCTTA
 AATTGGTTGACTTCTGCGCAATTGATCTCTGACGCTGACAACACTTA
 "CAT" box
 -40 -20 +1
 GGAAATGAAGAGATAAATGATGGGAACCCAGGCGGCTGC-CAGAGC
 GGAAATGAAGGTATAAATGTGAGTTACGGCAGTGGCTGCAGCAGATG
 "TATA" box

Fig. 4 Comparisons between the rat and human growth hormone-releasing factor precursor and gene sequences. **a**, Comparison of the deduced amino-acid sequences of the complete rat and human GHRF precursor proteins. Identical residues are shaded; several gaps have been inserted to maximize the alignment. The GHRF peptide sequence is underlined and residues thought to be involved in the processing of this peptide from the precursor are boxed. **b**, Comparison of the nucleotide sequences in the 5'-flanking region of the human and rat *ghrf* genes. Identical nucleotides are shaded; several gaps have been inserted to maximize the alignment. The positions of the consensus CAT and TATA box sequences are indicated.

between the entire coding regions and ~58% identity between both the 5- and 3'-non-translated sequences. We presume that this high conservation of non-coding sequences in the 5'-flanking region reflects a functional importance, perhaps in the regulation of *ghrf* gene expression.

Because GHRF is believed to be an important physiological regulator of growth hormone synthesis and secretion³⁻⁶, analysis of its synthesis and actions can be expected to lend insight into mechanisms by which the neural and hormonal control of somatic growth are integrated. The isolation of rat *ghrf* cDNA and genomic clones will enable us to use an animal model system to pursue questions concerning the expression of the *ghrf* gene in rat hypothalamus, to study its regulation by neural and hormonal influences, and to define the DNA sequences responsible for expression and regulation of this gene.

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- Spies, J., Rivier, J. & Vale, W. *Nature* **303**, 532-534 (1983).
- Merchenthaler, I., Vigh, S., Schally, A. & Petrusz, P. *Endocrinology* **114**, 1082-1085 (1984).
- Barinaga, M. et al. *Nature* **306**, 84-85 (1983).
- Glick, G. G. et al. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1553-1555 (1984).

5. Wehrenberg, W. B. *et al. Biochem. biophys. Res. Commun.* **109**, 382-387 (1982).
6. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J. & Rivier, J. *Endocrinology* **112**, 1553-1555 (1983).
7. Wehrenberg, W. B., Bloch, B. & Phillips, B. J. *Endocrinology* **115**, 1218-1220 (1984).
8. Gubler, U. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4311-4314 (1983).
9. Mayo, K. E., Vale, W., Rivier, J., Rosenfeld, M. G. & Evans, R. M. *Nature* **306**, 86-88 (1983).
10. Mayo, K. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 63-67 (1985).
11. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
12. Bloch, B. *et al. Nature* **301**, 607-608 (1983).
13. Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J. & Tager, H. S. *Ann. N.Y. Acad. Sci.* **343**, 1-16 (1980).
14. Bradbury, A. F., Finnie, M. D. A. & Smyth, D. G. *Nature* **298**, 686-688 (1982).
15. Ling, N. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4302-4306 (1984).
16. Sargent, T. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3256-3260 (1979).
17. Breathnach, R. & Chambon, P. A. *Rev. Biochem.* **50**, 349-383 (1981).
18. Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. *Nucleic Acids Res.* **8**, 127-142 (1980).
19. Corden, J. *et al. Science* **209**, 1406-1414 (1980).
20. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211-214 (1976).
21. Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276-278 (1982).
22. Guilleman, R. *et al. Science* **218**, 585-587 (1982).
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
24. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).

Clusters of CpG dinucleotides implicated by nuclease hypersensitivity as control elements of housekeeping genes

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DNA sequences of the X-chromosome-linked hypoxanthine phosphoribosyltransferase (HPRT) and glucose 6-phosphate dehydrogenase (G6PD) genes have revealed the presence of clusters of CpG dinucleotides¹⁻³, raising the possibility that such clusters are involved in the control of expression of these genes, which are expressed in all tissues. Although CpG clusters are not exclusive features of the X chromosome⁴⁻⁶, the analysis of X-linked genes provides the means to determine whether CpG clusters are control elements; one of the two homologous X loci in female mammals is not expressed⁷, so that active and inactive versions of the gene can be compared. In fact, it has been shown that these CpG clusters are undermethylated when the gene is active and extensively methylated when the gene is inactive^{2,8}. In addition to hypomethylation, chromatin hypersensitivity to endonuclease digestion is a known hallmark of regulatory sequences in eukaryotic genes⁹. We report here that the CpG clusters of the active *hpert* and *g6pd* genes are not only undermethylated, but also hypersensitive to *MspI*, DNase I and *S₁* nuclease, further supporting the suggestion that they are involved in the control of expression of these genes.

Figure 1a shows a restriction enzyme map of the 5' region of the human *hpert* locus. A notable feature of the promoter region is a dense cluster of CpG dinucleotides (12 *HpaII* and 15 *HhaI* sites within a 1.0-kilobase (kb) segment)². As probe for studies of the *hpert* locus, we have used an intron sequence that hybridizes only to the *hpert* locus that is on the human X chromosome (pPB1.7)^{1,2}.

Figure 2a shows that the CpG cluster from the active *hpert* gene is extremely sensitive to digestion with *MspI*, whereas the cluster from the inactive gene is not. When chromatin of male lymphoblasts (XY) is digested for 20 min with only 0.1 U of *MspI* per µg DNA, the single active gene is completely cleaved, as shown by the presence of only the 9.5-kb sub-band in *EcoRI* digests of the purified DNA; this sub-band is also seen in chromatin digests from female lymphocytes. However, the 11.5-kb uncleaved fragment is also present, even if 10 times more *MspI* is used. We infer that the uncleaved fragment comes from the inactive X because it is found exclusively in females and because the intensity of the band is proportional to the number of inactive X chromosomes (Fig. 2a, XX, XXX and XXXX

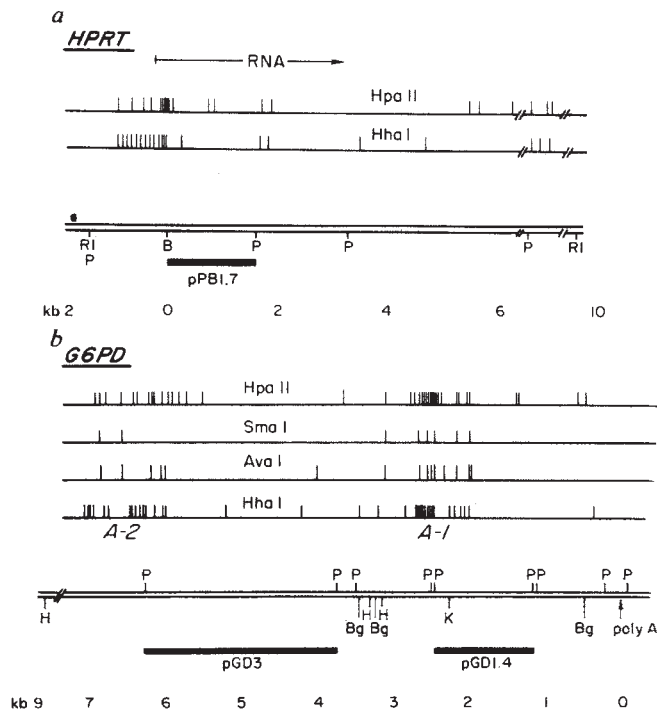


Fig. 1 Map showing relevant regions of *hpert* (a) and *g6pd* (b) loci and the cloned sequences used as probes. B, *BamHI*; Bg, *BglI*; RI, *EcoRI*; H, *HindIII*; K, *KpnI*; P, *PstI*.

lymphocytes). Further evidence that the inactive gene is resistant comes from studies of mouse/human hybrid cells, which contain the human inactive X homologue but not the active one. These hybrids are also a source of reactants that have activated the *hpert* gene on the inactive X. Our observations of these reactants indicate that acquisition of expression is accompanied by acquisition of nuclease hypersensitivity. When the gene is inactive (that is, on the isolated inactive X), the CpG cluster is resistant to *MspI* digestion (Fig. 2b). However, when the *hpert* locus is re-expressed² as a result of reactivation of the locus on the inactive X, either spontaneously (X Rea-1) or induced by 5-azacytidine (X Rea-2), the cluster becomes as sensitive to *MspI* as that on the active X (Fig. 2b).

The 5' *hpert* cluster is hypersensitive to digestion not only by *MspI*, but also to DNase I and *S₁* nuclease (Fig. 3). Moreover, the region of sensitivity not only coincides with the cluster, but corresponds to the region within the cluster which contains the greatest number of CpGs. The fragments resulting from cleavage of chromatin by DNase I and *S₁* (and DNA by *PstI*) are slightly larger than the *BamHI/PstI* fragment obtained by cleaving purified DNA (Fig. 3). Therefore, the hypersensitive site is immediately 5' to the *BamHI* site (see Fig. 1).

We have examined CpG clusters from *g6pd*, another X-linked housekeeping gene. It is not known if there are 5' CpG clusters at this locus because clones from this region have not been isolated. However, there are two extensive clusters in the 3' coding region. Figure 1b shows a map of the 3' region and the two genomic probes³ used for the analysis. In chromatin from the active gene, either in male cells (not shown) or in hybrids, both clusters are extremely sensitive to *MspI* digestion. Sensitivity of the A-1 cluster in the active gene is shown in Fig. 4. In chromatin from female cells, not all clusters are cleaved by *MspI*, as we observe the full-length *BglI* fragment in addition to the cleavage product obtained from active genes (Fig. 4). Studies of hybrid cells confirm that resistant clusters are from the inactive X; chromatin of hybrids with the inactive gene, but not the active one, is resistant to *MspI* (Fig. 4).

The A-1 and A-2 clusters are also hypersensitive to DNase I and *S₁* nuclease (Fig. 5). Moreover, as occurs with *hpert*, the

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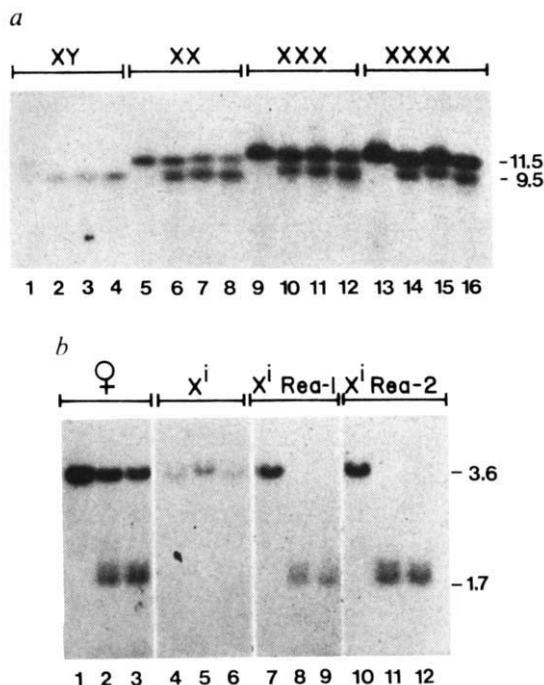


Fig. 2 *MspI* digests of chromatin, showing sensitivity of the clusters in active *hprt* genes and resistance in inactive ones. **a**, Lymphoblasts. Lanes 1–4, 46 XY; lanes 5–8, 46 XX; lanes 9–12, 46 X/isodicentric X; lanes 13–16, 48 XXXX. Samples were treated with 0, 0.1, 0.3 or 1.0 U *MspI* per μg DNA (from left to right in each group). Purified DNA was cut with *EcoRI* and probed with pPB1.7. **b**, Fibroblasts. Lanes 1–3, human 46 XX; lanes 4–6, hybrid G1 $\times$ A9 with inactive human X; lanes 7–9, X Rea-1, spontaneous *hprt* reactant of G1 $\times$ A9; lanes 10–12, X Rea-2, 5-azacytidine-induced *hprt* reactant of G1 $\times$ A9. The hybrids and reactants have been described elsewhere². The hybrids and reactants have been described elsewhere². The hybrids and reactants have been described elsewhere². DNA was cut with *PstI* and probed with pPB1.7. Numbers on the right refer to molecular size markers in kb.

Methods. Chromatin was obtained from nuclei of lymphoblasts and fibroblasts. Lymphoblast cultures, collected at a density of 10^6 cells ml^{-1} , were washed in 0.9% saline and suspended (3×10^7 cells ml^{-1}) in RSB (10 mM NaCl, 3 mM MgCl_2 , 10 mM Tris-HCl pH 7.5) supplemented with 1 mM phenylmethylsulphonyl fluoride (PMSF) and lysed by adding NP40 to a concentration of 0.2%. Nuclei from human dermal fibroblasts (or human/mouse hybrids) were purified as follows. The cells were trypsinized and resuspended in growth medium with 15% fetal calf serum, washed with 0.9% saline and treated with RSB for 10 min at 4°C. Triton X-100 was added (to 0.2%) and the cells were lysed by Dounce homogenization to release the nuclei; then PMSF was added to a concentration of 1 mM. Nuclei were purified by washing in 50 ml RSB with 0.1 mM PMSF and resuspension in the same buffer so as to bring the concentration of nucleic acid to 250–500 μg ml^{-1} . Incubations with nuclease were carried out for 20 min at 37°C. Reactions were stopped by quickly bringing the samples to 0°C. The nuclei were pelleted and resuspended in 0.15 M NaCl, 0.01 M EDTA, 0.1 M Tris-HCl pH 7.5 and the DNA was purified by standard procedures².

hypersensitive regions correspond to regions having the greatest concentration of CpGs. For example, the A-2 cluster spans a 1.5-kb region, but the region of DNase I and S_1 hypersensitivity is less extensive, ~0.4 kb, deduced from the broad band (3.7–4.1 kb) representing the cleavage products (Fig. 5c, d). Furthermore, the absence of detectable DNase I or S_1 cleavage products in *PstI* digests of the DNA probed with pGD3 (not shown) indicates that the sensitive region does not extend very far into the pGD3 sequence. That the hypersensitive region is confined to the densest part of the cluster is even more apparent in the A-1 cluster, for here there are two well-separated cleavage products (Fig. 5a); the hypersensitive sites map to the densest parts

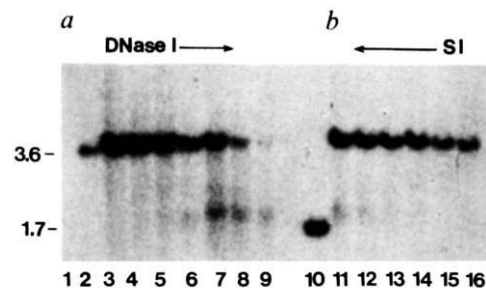


Fig. 3 DNase I and S_1 nuclease digests of chromatin showing hypersensitivity of the *hprt* 5' CpG cluster in active genes. Lymphoblasts (46 XY) were prepared as described in Fig. 2 legend. For S_1 digests, nuclei were suspended in S_1 buffer (3 mM ZnCl_2 , 30 mM NaCl, 0.2 mM EDTA, 30 mM sodium acetate pH 4.5) rather than RSB. Samples were incubated with increasing amounts of DNase I (0–0.02 U per μg DNA; lanes 1–9) or S_1 (0–20 U per μg DNA; lanes 10–11) for 20 min. DNA was cut with *PstI* and probed with pPB1.7. A *BamHI/PstI* digest of placental DNA is included as an internal marker (lane 10).

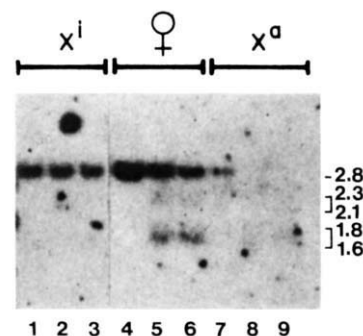


Fig. 4 *MspI* digest of chromatin, showing sensitivity of the A-1 CpG cluster in active *g6pd* genes but resistance in inactive ones. Nuclei were prepared and digested with *MspI* as described for Fig. 2. Lanes 1–3, human/mouse hybrid G1 $\times$ A9 with inactive human X; lanes 4–6, 46 XX human fibroblast; lanes 7–9, human/mouse hybrid E $\times$ A9 with active X. Each sample was treated with 0, 0.5 or 1.5 U *MspI* per μg DNA. DNA was cut with *BglI* and probed with pGD1.4.

of the CpG cluster whereas the intervening region, which is relatively free of CpG dinucleotides, is insensitive.

Although both A-1 and A-2 clusters exhibit nuclease hypersensitivity, there are subtle differences between them. The relative intensity of the cleaved fragments indicates that the A-1 cluster is less sensitive than the A-2 cluster to either DNase I or S_1 (Fig. 5).

Our observations support the hypothesis that CpG clusters are involved in regulating the expression of *hprt* and *g6pd*. Like all regulatory regions identified⁹, these clusters are hypersensitive to nuclease digestion and this sensitivity is concordant with expression of the relevant gene. Moreover, for both loci, the region of hypersensitivity maps to the clusters themselves rather than merely nearby, especially remarkable because DNase I usually prefers A+T-rich sequences¹⁰.

The molecular determinants of nuclease hypersensitivity have been shown to be heterogeneous^{9,11–13} and are likely to be complex. The ability to examine these CpG clusters in active and inactive homologues within the same cell provides insights into mechanisms responsible for hypersensitivity, at least at the loci examined. The differential nuclease sensitivity of homologous sites within the same cell indicates that neither *trans*-acting proteins¹¹ nor DNA sequences are sufficient determinants. Another component that seems to be required is hypomethylation of these clusters. We have never observed discordance between hypomethylation, nuclease sensitivity and expression of the relevant locus (refs 7, 8 and this paper).

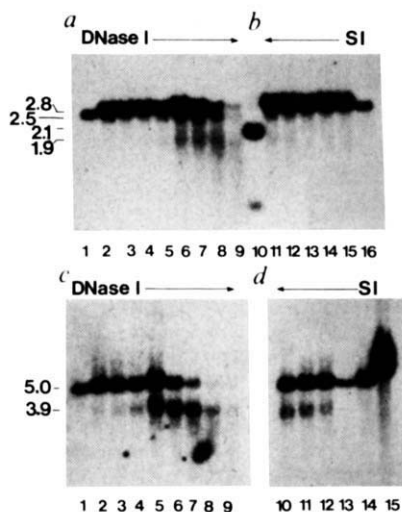


Fig. 5 DNase I (*a, c*) and S_1 nuclease (*b, d*) digests of chromatin showing sensitivity of both A-1 and A-2 CpG clusters in active *g6pd* genes. The nuclease digests of chromatin from 46 XY lymphoblasts were the same as used in Fig. 3. DNA was cut with *Bgl*II and probed with pGD1.4 (*a*, DNase I; *b*, S_1) or cut with *Hind*III and probed with pGD3 (*c*, DNase I; *d*, S_1). A *Bgl*II/*Kpn*I digest of placental DNA (lane 10 in *a, b*) is the internal marker. Lanes in *a* correspond exactly to those in Fig. 3; lanes 10–15 in *d* correspond to lanes 11–16 in Fig. 3.

The function of CpG clusters in these housekeeping genes is unknown. Although hypersensitivity of the 5'-promoter region in an active gene is not surprising, this region in *hprt* is atypical (ref. 14 and D. J. Jolly, personal communication), lacking the characteristic regulatory signals (TATA and CAT). Thus, the 5' cluster may provide a novel regulatory mechanism. The explanation for hypersensitivity in the 3' region of *g6pd* is more enigmatic; however, the resemblance to the *hprt* 5' cluster suggests that 3' clusters may also be regulatory elements. The explanation for hypersensitivity of these CpG clusters provides the means to identify similar sequences elsewhere in the genome and the location of related clusters may provide insights into function. In any event, it is clear that CpG clusters are not unique features of X chromosomes, as they have been observed in autosomal genes^{4,6}. On the other hand, these clustered CpG dinucleotides have been found most frequently in genes that are constitutively expressed, so that they may be special attributes of housekeeping genes. Such genes are unique because they are functional in all cells of the organism. The CpG-rich clusters might provide a cooperative means of minimizing the effects of chance *de novo* methylation in the regulatory regions, and/or by altering DNA structure. In conjunction with hypomethylation, these clusters could serve as irreversible switches to maintain the continuous activity of housekeeping genes.

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Diacylglycerol in large α -actinin/actin complexes and in the cytoskeleton of activated platelets

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The interaction of the cytoskeleton with plasma membranes may be mediated by vinculin^{1–4}, α -actinin^{5,6} and other proteins^{7–11}; α -actinin can interact specifically with model membranes only if they contain diacylglycerol and palmitic acid¹². On stimulation of platelets by thrombin, which leads to a reorganization of the cytoskeleton^{13–15}, diacylglycerol is produced rapidly¹⁶, simultaneously with the disappearance of phosphatidylinositol^{16–19}. One important function of the diacylglycerol produced in platelets may be the activation of the Ca^{2+} - and phospholipid-dependent protein kinase C^{20–22}. We show here that, in the presence of diacylglycerol and palmitic acid, a supramolecular complex between α -actinin and actin is formed *in vitro*. In the electron microscope, this complex displays substructures similar to those of microfilament bundles *in vivo*. Furthermore, such α -actinin/lipid complexes can also be formed *in situ* during the stimulation of blood platelet aggregation. Thus, α -actinin may be one of the proteins directly involved in structures connecting the cytoskeleton to cell membranes.

The protein α -actinin is associated with the cytoskeleton in many cells^{5,23–25}, including platelets^{26–29}. This association is particularly prominent on physiological activation of platelets

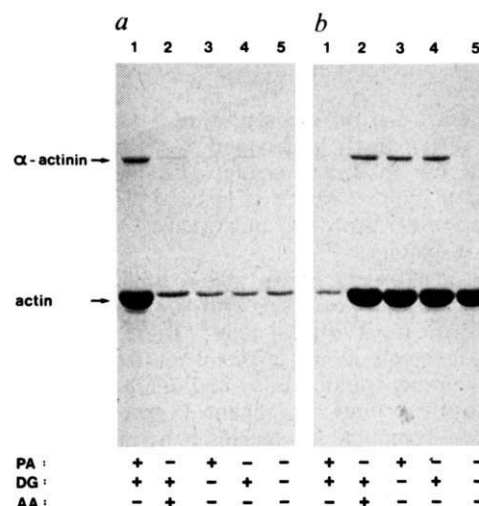


Fig. 1 SDS-polyacrylamide gel electrophoresis of α -actinin/lipid complexes bound to filamentous actin. *a*, 10,000g pellets; *b*, 120,000g pellets. *a, b*, Lane 1, α -actinin and actin incubated with palmitic acid (PA) and diacylglycerol (DG); lane 2, with arachidonic acid (AA) and DG; lane 3, with fatty acid only; lane 4, with DG only; lane 5, actin incubated without lipids. *a*, Lane 1, >90% of the protein was in the 10,000g pellet; *b*, lane 1, <10% was in the 120,000g pellet. In all control experiments where the two specific lipids were absent the opposite result was observed, that is, <10% of the protein was in the 10,000g pellet and >90% in the 120,000g pellet.

Methods. 7 μ g α -actinin (isolated from chicken gizzard) was pre-incubated with appropriate lipids for 45 min at room temperature. 55 μ g G-actin (or F-actin) was then added and polymerization initiated by adding $MgCl_2$ (10 mM final concentration) and KCl (150 mM final concentration). After 30 min at room temperature, the samples were centrifuged for 5 min at 10,000 g. The 10,000g supernatants were removed and recentrifuged in a Beckman airfuge at 120,000g; <2% protein was left in this supernatant. Both pellets were washed and analysed on an 0.1% SDS/7.5% polyacrylamide gel³⁴.

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- Jolly, D. J., Esty, A. C., Bernard, H. U. & Friedmann, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5038–5041 (1982).
- Wolf, S. F., Jolly, D. J., Lunnen, K. D., Friedmann, T. & Migeon, B. B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2806–2810 (1984).
- Toniolo, D. *et al.* *EMBO J.* **3**, 1987–1995 (1984).
- Stein, R., Sciaky-Gallili, N., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4035–4039 (1983).
- Chen, M.-J. *et al.* *J. biol. Chem.* **259**, 3932–3943 (1984).
- Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. *Cell* **37**, 381–391 (1984).
- Lyon, M. F. *Biol. Rev.* **47**, 1–35 (1974).
- Wolf, S. F. *et al.* *Nucleic Acids Res.* **12** (24), 9333–9348 (1984).
- Weisbrod, S. *Nature* **297**, 289–295 (1982).
- Dingwall, C., Lomonosoff, G. P. & Laskey, R. A. *Nucleic Acids Res.* **9**, 2659–2673 (1981).
- Emerson, B. S. & Felsenfeld, G. *Proc. natn. Acad. Sci. U.S.A.* **81**, 95–99 (1984).
- Jongstra, J. *et al.* *Nature* **307**, 708–714 (1984).
- Schubach, W. & Groudine, M. *Nature* **307**, 702–708 (1984).
- Melton, D. W., Konecki, D. S., Brennan, J. & Caskey, C. T. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2147–2151 (1984).

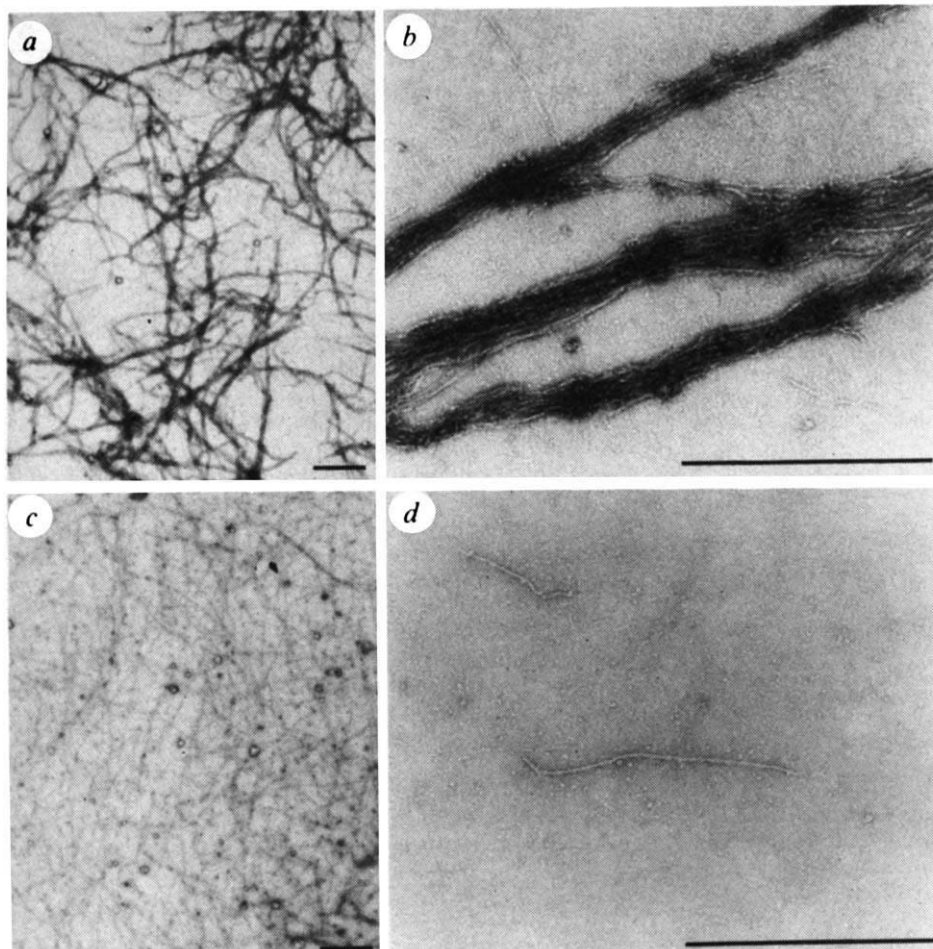


Fig. 2 Electron micrographs of negatively stained preparations of actin filaments *a, b*, with α -actinin, diacylglycerol and palmitic acid or *c, d*, with α -actinin alone. Scale bars, 0.5 μ m.

Methods. Reaction mixtures containing actin, α -actinin, diacylglycerol and palmitic acid (*a, b*) or actin and α -actinin without lipids (*c, d*) were prepared and polymerized as described in Fig. 1 legend. Carbon-coated collodion grids were glow discharged and placed on a drop of particle suspension for adsorption. Samples *a* and *c* were diluted to 200 μ g protein per ml immediately before being spread onto grids; samples *b* and *d* were centrifuged for 5 min at 10,000g as described in Fig. 1 legend. The 10,000g pellets were then washed twice with buffer before being spread onto grids. Adsorption of proteins was followed by post-treatment with the same buffer solution, rinsing with distilled water and staining with 2% uranyl acetate (pH 4.2). Specimens were examined in a Zeiss 109 electron microscope.

with thrombin²⁶, but the association of α -actinin with the cell membrane is less clearly understood. Some studies suggest that α -actinin is not in direct contact with the membrane^{3,30,31}, whereas others indicate that it is in close association with the cellular membranes of various nucleated cells^{5,6} and the plasma membrane of platelets^{26,32}.

Recently, it has been shown that α -actinin forms a highly specific stoichiometric complex with two lipid components of the entire lipid extract of yeast cells¹². These complexes always contain one molecule of diacylglycerol, one fatty acid of a certain type (for example, palmitic acid) and one α -actinin molecule. The need for these three components is absolute and has been demonstrated in monolayers lacking either of the two lipids¹².

Our results suggest that fatty acids, together with the diacylglycerol formed during thrombin activation of platelets, interact with α -actinin and may affect its location either in or at the plasma membrane, as well as its association with cytoskeletal actin filaments. Diacylglycerol may serve as a signal for the transmembrane control of protein phosphorylation via the activation of a novel protein kinase^{21,22}. Similarly, diacylglycerol and fatty acids formed in the membrane during platelet activation may serve as a transmembrane signal for the redistribution of α -actinin in membrane regions of the cell and thereby the cytoskeleton.

A tight complex is formed between α -actinin, diacylglycerol and palmitic acid (but not between α -actinin diacylglycerol and arachidonic acid)¹². Here we show, first, that this complex formation leads to large aggregates of α -actinin (from smooth muscle) and actin of the type observed in actin filament bundles, and second, that this complex is actually formed *in situ* during platelet activation.

The addition of α -actinin, diacylglycerol and palmitic acid to filamentous actin results in binding of the tri-component complex as well as aggregation of these actin/tri-component complexes. These large aggregates form a pellet when spun at

10,000g when the appropriate lipids, such as palmitic acid and 1,2-diolelin, are present (Fig. 1, lane 1). Similar incubations of actin and α -actinin with other components (see Fig. 1) result in no such aggregation. This suggests that reorganization of the filamentous actin into larger complexes *in vitro* takes place only in the presence of the α -actinin/palmitic acid/diacylglycerol complex and is not just a general lipid effect. Similar results were obtained when the complex was first added to globular (G) actin followed by the induction of polymerization.

Electron micrographs (Fig. 2) confirmed these sedimentation results. A network of actin filament bundles was observed in the presence of diacylglycerol and palmitic acid (Fig. 2a)) whereas in the absence of lipids the network contained only single filaments (Fig. 2c). The 10,000g pellets of these preparations collected in the presence of lipids showed large actin filament bundles (10–40 filaments), whereas pellets formed in the absence of the appropriate lipids displayed an occasional single filament (Fig. 2b, d, respectively).

These results suggest that the formation of diacylglycerol and the appropriate fatty acid in membranes and their subsequent binding to a fraction of α -actinin affect the microfilaments in the cytoskeleton. To confirm whether such a complex is formed *in situ* and to demonstrate its biological significance, we studied its formation in blood platelets.

Washed human platelets were labelled with ³H-palmitic or ³H-arachidonic acid, activated with thrombin and the cytoskeleton of resting and activated cells was isolated. The binding of lipid components to the cytoskeleton of activated platelets labelled with palmitic acid is six times higher than that of control platelets labelled with arachidonic acid. The relative binding of lipids to the cytoskeleton of activated palmitic acid labelled platelets is even more significant, considering the higher incorporation of arachidonic acid into resting platelets (74% of the total added, compared with 40% for palmitic acid), and also the higher incorporation of arachidonic acid into phospholipids

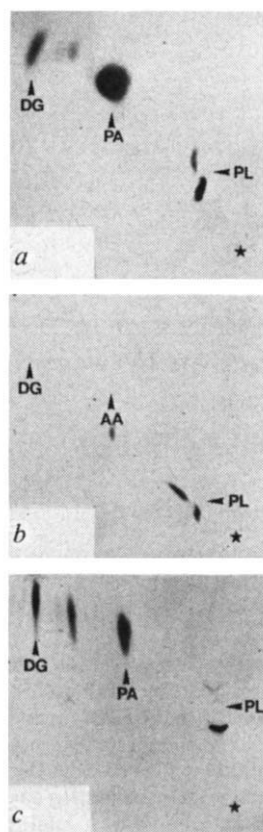


Fig. 3 Autoradiograms of lipids extracted from cytoskeletons or from immunoprecipitated α -actinin from cytoskeletons of thrombin-activated platelets analysed by two-dimensional TLC. *a*, Lipids extracted from cytoskeletons of ^3H -palmitic acid labelled and thrombin-activated platelets; *b*, lipids extracted from cytoskeletons of ^3H -arachidonic acid labelled and thrombin-activated platelets; *c*, lipids extracted from material immunoprecipitated by anti- α -actinin rabbit antiserum from dissolved cytoskeletons of ^{14}C -palmitic acid labelled and thrombin-activated platelets.

Methods. Platelets were prepared by a modified procedure of Schmidt and Rasmussen^{27,33}. The washed platelets (3×10^8 cells ml^{-1}) were incubated at 23°C with ^3H -palmitic acid or ^3H -arachidonic acid ($30 \mu\text{Ci ml}^{-1}$) in Ringer/citrate/dextrose buffer (RCD) (1.6 mM CaCl_2 , 3.9 mM KCl, 107.8 mM NaCl, 0.5% glucose and 0.62% trisodium citrate, pH 7.4) for 2 h. The cells were centrifuged at $10,000g$ for 4 min and the pellet washed three times with RCD buffer. Cytoskeletons were isolated as described by Phillips *et al.*²⁸ and Rotman *et al.*²⁶. Thrombin-activation was performed with 1 unit ml^{-1} (final concentration) at 37°C . After 20 s, a solution containing Triton X-100 and EGTA was added to the platelet suspension until the concentrations of Triton X-100 and EGTA were 1% and 5 mM, respectively. The suspension was stirred for a further minute, kept on ice for 30 min, and then centrifuged for 4 min at $10,000g$. The supernatants were removed and the pellets washed twice with RCD buffer containing 1% Triton X-100 and 5 mM EGTA and once with RCD buffer only. The lipids were extracted from the cytoskeletons or the immunoprecipitated α -actinin (as described in Fig. 4 legend) by the method of Bligh and Dyer³⁵ and separated by two-dimensional chromatography¹² on high performance TLC plates (Merck). The first dimension (upward) was in chloroform/methanol/ H_2O (65:25:4). The second dimension (to the left) was in *n*-hexane/chloroform/methanol/diethylether (90:65:25:10). Radioactive profiles were obtained by exposing Kodak XS-5 X-ray film to the dried plates at -80°C for 7 days (*a*, *b*) or 1 day (*c*). Similar results were obtained when the cytoskeletons were prepared without Triton X-100. Here, the platelets were sonicated and the pellet ($1,000g$) used for immunoprecipitation and lipid analysis. Arrowheads, phospholipids (PL), palmitic acid (PA), arachidonic acid (AA) and diacylglycerol (DG). Asterisk, origin of chromatography.

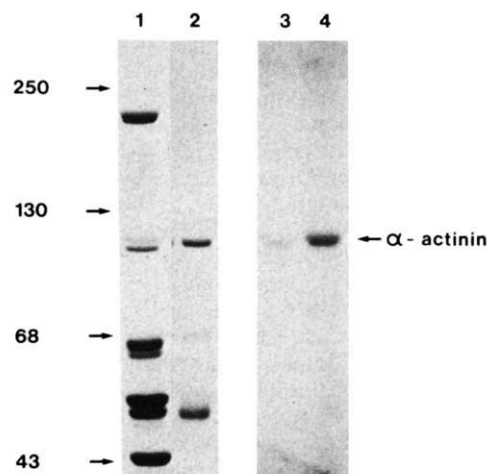


Fig. 4 Analysis of material immunoprecipitated by anti- α -actinin rabbit antiserum from cytoskeletons of thrombin-activated, ^3H -palmitic acid labelled platelets. (0.1% SDS/7.5% polyacrylamide gel electrophoresis). Lane 1, Coomassie brilliant blue staining of cytoskeletons (Triton X-100 residue) from thrombin-activated platelets. Lane 2, Coomassie brilliant blue staining of material immunoprecipitated by anti- α -actinin rabbit antiserum from cytoskeletons of thrombin-activated platelets. Lane 3, fluorogram of cytoskeletons (Triton X-100 residue) from ^3H -palmitic acid labelled and thrombin-activated platelets. Lane 4, fluorogram of material immunoprecipitated by anti- α -actinin rabbit antiserum from cytoskeletons of ^3H -palmitic acid labelled and thrombin-activated platelets. Numbers represent relative molecular mass, $\times 1,000$.

Methods. Platelets and cytoskeletons were prepared as described in Fig. 3 legend. For immunoprecipitation, isolated cytoskeletons from ^3H -palmitic acid labelled platelets were dissolved in 1% SDS and then heated for 3 min at 95°C . After dissociation in SDS, the sample was centrifuged for 4 min at $10,000g$. The clear supernatant fraction was diluted with TNET-buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100) to give final concentration of 0.1% SDS. The diluted suspension was pre-cleared with $100 \mu\text{l}$ of a 10% w/v suspension of glutaraldehyde-fixed *Staphylococcus aureus* cells for 45 min at room temperature. Then, $200 \mu\text{l}$ anti- α -actinin rabbit antiserum was added and the mixture gently shaken for 16 h at 4°C . Immune complexes were adsorbed with a 10% w/v suspension of glutaraldehyde-fixed *S. aureus*³⁶ for 60 min at room temperature, washed, eluted and analysed³⁴. For fluorography, the gel was soaked in 200 ml of 1 M sodium salicylate³⁷ for 20 min immediately after electrophoresis and exposed for 4 weeks. To minimize any loss of ^3H -palmitate label, SDS-polyacrylamide gels were not fixed and stained after electrophoresis³⁸.

for the same time interval (90% of the total uptake, compared with 3% for palmitic acid).

The relative increase of radioactive lipids in the cytoskeleton of activated platelets (labelled with ^3H -palmitic acid) compared with that of resting platelets was at least 30-fold, indicating that physiological activation leads to a sizeable and specific attachment of lipids to the cytoskeletal elements. The low binding of radioactive lipids to the cytoskeleton of resting platelets may result from a small population of activated platelets in the preparation.

Cytoskeletons from activated platelets pre-labelled by either ^3H (or ^{14}C) palmitic acid or ^3H -arachidonic acid were isolated and the radioactively labelled lipids attached to the cytoskeletons analysed by high performance TLC (Fig. 3). Most of the radioactivity attached to the cytoskeleton of palmitic acid labelled platelets came from palmitic acid, diacylglycerol and phospholipids (Fig. 3*a*). The ratio of labelled palmitic acid to diacylglycerol was not 1:1, probably because some of the palmitic acid was attached to other cytoskeletal elements than α -actinin. In arachidonic acid labelled platelets, the radioactivity associated with the cytoskeleton was mainly in phospholipids, less in free diacylglycerol and practically none in free fatty acid (Fig. 3*b*). In this case, 90% of the total radioactivity

in the platelets were incorporated into phospholipids; however, the attachment of labelled lipids to the cytoskeleton was sixfold less than that of palmitic acid labelled platelets.

Further evidence that the lipids in the cytoskeleton are bound to α -actinin was obtained by the immunoprecipitation of α -actinin from solubilized cytoskeletons of ^{14}C -palmitic acid labelled cells by anti- α -actinin antibodies. Analysis of the lipids attached to this immunoprecipitate by two-dimensional high performance TLC shows that mainly diacylglycerol and palmitic acid are attached to the α -actinin (Fig. 3c), the ratio of these lipids being almost 1:1. Furthermore, this precipitate, when analysed by SDS-polyacrylamide gel electrophoresis and fluorography, shows a clear band of radioactivity in the position of α -actinin (Fig. 4). Gel filtration and chromatofocusing give further evidence of tight binding of radioactive lipid components to protein with relative molecular mass and isoelectric point values similar to α -actinin (data not shown).

Our results show that at least 30-fold more lipids (diacylglycerol and palmitic acid) are attached to the cytoskeleton during platelet activation and that diacylglycerol is associated tightly with α -actinin immunoprecipitated from cytoskeletons of activated platelets. Neither the molecular mechanism nor the significance of this process are yet understood. Thrombin, through interaction with its membrane receptor, may initiate the cleavage of phosphatidylinositol to diacylglycerol as well as of phospholipids to fatty acids via activation of phospholipases. The mechanism by which the phospholipases are activated is not yet known. If the appropriate fatty acid is available, a stable complex will be formed between it, diacylglycerol¹⁶⁻¹⁹ and the α -actinin¹² that is already associated with the membrane²⁶, or may now be capable of association with the membrane. At the same time, the thrombin-induced activation leads to rapid actin polymerization and reorganization of the cytoskeleton^{13,27}. Such a complex of diacylglycerol, fatty acid and α -actinin can increase the affinity and attachment of membrane-associated α -actinin with the cytoskeleton²⁶ and is therefore fundamental in the interaction between the cytoskeleton and the membrane. In addition, the interaction of α -actinin/lipid complexes among themselves, suggested by the monolayer complexes found¹², may initiate bundling of actin filaments in membranes. Provided that the appropriate lipids are available, a similar mechanism may be involved in restructuring microfilaments in the cytoplasm. As none of the components (diacylglycerol, fatty acids, α -actinin and actin) is unique to platelets but all are present in many other cell systems, this mechanism could be general in cytoskeleton/membrane interaction in cell-shape changes and migration.

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- Geiger, B. *Cell* **18**, 193-205 (1979).
- Burridge, K. & Feramisco, J. R. *Cell* **19**, 587-595 (1980).
- Geiger, B., Tokuyasu, K. T., Dutton, A. H. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4127-4131 (1980).
- Jokusch, B. M. & Isenberg, G., *Proc. natn. Acad. Sci. U.S.A.* **78**, 3005-3009 (1981).
- Lazarides, E. & Burridge, K. *Cell* **6**, 289-298 (1975).
- Jokusch, B. M. *et al. Nature* **270**, 628-629 (1977).
- Maher, P. & Singer, S. J. *Cell Motil.* **3**, 419-429 (1983).
- Oesch, B. & Birchmeier, W. *Cell* **31**, 671-679 (1982).
- D'Angelo Siliciano, J. & Craig, S. W. *Nature* **300**, 533-535 (1982).
- Burridge, K. & Connell, L. J. *Cell Biol.* **97**, 359-367 (1983).
- Repasky, E. A., Granger, B. L. & Lazarides, E. *Cell* **29**, 821-833 (1982).
- Meyer, R. K., Schindler, H. & Burger, M. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4280-4284 (1982).
- Jennings, L. K., Fox, J. E. B., Edwards, H. H. & Phillips, D. R. *J. biol. Chem.* **256**, 6927-6932 (1981).
- Carlsson, L., Markey, F., Blikstad, I., Persson, T. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6376-6380 (1979).
- Nachmias, V. T. *J. Cell Biol.* **86**, 795-802 (1980).
- Bell, R. L. & Majerus, P. W. *J. biol. Chem.* **255**, 1790-1792 (1980).
- Rittenhouse-Simmons, S. J. *clin. Invest.* **63**, 580-587 (1979).
- Lapetina, E. G. *Trends Pharmac. Sci.* **3**, 115-118 (1982).
- Lapetina, E. G. & Cuatrecasas, P. *Biochim. biophys. Acta* **573**, 394-402 (1979).
- Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. *J. biol. Chem.* **255**, 2273-2276 (1980).
- Nishizuka, Y. *Nature* **308**, 693-698 (1984).
- Michell, B. *Trends biochem. Sci.* **8**, 263-265 (1983).

- Lazarides, E. J. *Cell Biol.* **68**, 202-219 (1976).
- Webster, R. E., Osborn, M. & Weber, K. *Expl. Cell Res.* **117**, 47-61 (1979).
- Feramisco, J. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3967-3971 (1979).
- Rotman, A., Heldman, J. & Linder, S. *Biochemistry* **21**, 1713-1720 (1982).
- Pribluda, V. & Rotman, A. *Biochemistry* **21**, 2825-2832 (1982).
- Phillips, D. R., Jennings, L. K. & Edwards, H. H. *J. Cell. Biol.* **86**, 77-86 (1980).
- Langer, B. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 432-435 (1982).
- Geiger, B., Dutton, A. H., Tokuyasu, K. T. & Singer, S. J. *J. Cell Biol.* **91**, 614-628 (1981).
- Burridge, K. & McCullough, L. J. *J. Supramolec. Struct.* **13**, 53-65 (1980).
- Debus, E., Weber, K. & Osborn, M. *Eur. J. Cell Biol.* **24**, 45-52 (1981).
- Schmidt, K. G. & Rasmussen, J. W. *Scand. J. Haemat.* **23**, 88-96 (1979).
- Lämml, U. K. *Nature* **227**, 680-685 (1970).
- Bligh, E. G. & Dyer, W. I. *Can. J. biochem. Biophys.* **37**, 911-917 (1959).
- Kessler, S. W. *J. Immun.* **115**, 1617-1624 (1975).
- Chamberlin, J. P. *Analyt. Biochem.* **98**, 132-135 (1979).
- Schlesinger, M. S., Magee, A. I. & Schmidt, M. F. *G. J. biol. Chem.* **255**, 10021-10024 (1980).

Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin

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There is evidence that the polymerization of actin takes place at the plasma membrane¹, and that profilactin (profilin/actin complex), the unpolymerized form of actin found in extracts of many non-muscle cells, serves as the immediate precursor^{2,3}. Both isolated profilin and profilactin interact with detergent when analysed by charge shift electrophoresis, indicating that they have amphipathic properties and may be able to interact directly with the plasma membrane⁴. We demonstrate here that isolated profilin, as well as the profilactin complex, interacts with anionic phospholipids. Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) was found to be the most active phospholipid, causing a rapid and efficient dissociation of profilactin with a concomitant polymerization of the actin in appropriate conditions. These and other observations suggest the possibility of a relationship between the induction of actin filament formation and the increased activity in the phosphatidylinositol cycle seen as a result of ligand-receptor interactions in various systems.

Many eukaryotic cells have a dense weave of microfilaments (actin filaments plus associated proteins) in close association with the plasma membrane⁵, apparently lying beneath the lipid bilayer all around the cell^{6,7}. The dramatic changes in surface morphology and motile behaviour often seen in response to growth hormones and other cell stimulating agents seem to reflect changes in the organization and activity of this actin filament system⁸, suggesting that regulated polymerization and crosslinking of actin and subsequent translocation (possibly myosin-linked) of filament assemblies formed are important steps in cell motility⁶. The detailed molecular mechanisms involved in these steps and their control, however, are unclear. Neither the site of polymerization of actin nor the nature of the transmembrane signal(s) inducing filament formation has been identified. The fact that actin polymerization occurs at the plasma membrane suggests that the precursor form of actin may be near, and possibly interacts with, the lipid bilayer. As profilin and profilactin behave like membrane proteins on charge shift electrophoresis⁴, we have investigated the possibility that they interact directly with phospholipids using a viscosimetric assay that can detect effects on the stability of profilactin and on the kinetics and extent of actin polymerization.

Actin rapidly forms filaments when exposed to elevated salt concentrations (for example 50-100 mM KCl or millimolar concentrations of MgCl₂). However, mammalian profilin inhibits the polymerization of the homologous non-muscle actin (β - + γ -actin) quite efficiently in these conditions. Viscosimetric studies recently completed in our laboratory (H. Larsson, unpublished results) on the recombination of intact profilin and actin isolated from calf spleen⁴ have shown that in 50 mM KCl, 0.1 mM CaCl₂, 0.5 mM ATP, 0.5 mM dithiothreitol (DTT) (25 °C), the dissoci-

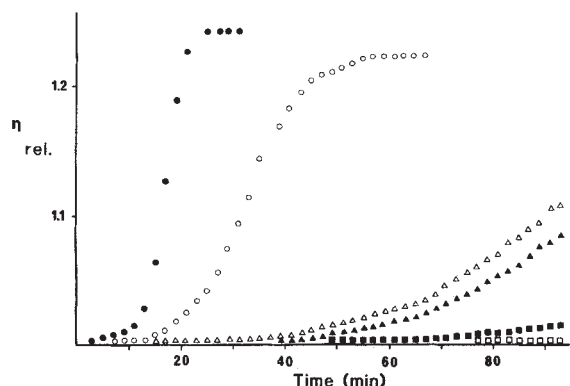


Fig. 1 Effect of various phospholipids on the stability of profilactin. Viscosity of profilactin was determined alone ($\square$) and in the presence of PtdIns(4,5)P₂, bovine brain ($\bullet$), PtdIns(4)P, bovine brain ($\circ$), PtdIns, pig liver (Δ), PhA, egg lecithin ($\blacktriangle$) and PtdS, bovine ($\blacksquare$) at various times of incubation. Phospholipid vesicles (100 μ l, final concentration 0.4 mg ml⁻¹), prepared by sonication in G-buffer (5 mM potassium phosphate buffer pH 7.6, 0.1 mM CaCl₂, 0.5 mM ATP, 10 μ M EDTA, 0.5 mM DTT) under nitrogen, were mixed with 429 μ l of G-buffer, 8.4 μ l of 1 mM EGTA, 14 μ l of 4 M KCl (final concentration 80 mM) and 177 μ l of profilactin- β in G-buffer (final concentration 0.4 mg ml⁻¹) in a Cannon-Manning viscometer at 35 °C (flow time 45 s). The viscosity of the incubation mixture was then measured at 2-min intervals for up to 2.5 h. In cases where polymerization had not occurred at the end of the incubation, the status of the actin was checked by adding MgCl₂ to 2 mM, which destabilizes profilactin. Filament formation then commences, with a lag phase of $\sim$ 10 min (K_d of profilactin in 1 mM MgCl₂ is 4×10^{-7} M; H. Larsson, unpublished result). All phospholipids were purchased from Sigma except pig liver PtdIns, which was obtained from Serdary, London, Canada.

ation constant (K_d) of the complex is $\leq 4 \times 10^{-8}$ M, and that in 1:1 mixtures of the two proteins no filament formation occurs. These and previous studies on the behaviour of calf spleen profilactin^{4,9,10} and on the kinetics of the polymerization of actin alone in various conditions formed the basis of our viscosimetric assay.

When profilactin- β (ref. 11) (0.4 mg ml⁻¹) was preincubated with phospholipid vesicles (0.8 mg ml⁻¹) for 15 min at low salt concentrations (5 mM potassium phosphate buffer pH 7.6, 0.5 mM ATP, 0.5 mM DTT) in the presence of 0.1 mM CaCl₂, then exposed to 80 mM KCl, all the anionic phospholipids tested destabilized the complex, causing variable amounts of the actin to polymerize. After preincubation with vesicles of phosphatidic acid (PhA) and PtdIns(4,5)P₂ the actin polymerized with a $t_{1/2}$ of 22 min. Phosphatidylserine (PtdS) was slightly less active, showing a $t_{1/2}$ of polymerization of 25 min, and PtdIns (soy bean) and phosphatidylglycerol (PtdG) were even less active ($t_{1/2}$ 35 and 37 min, respectively). The cationic phospholipids phosphatidylcholine (PtdC) and phosphatidylethanolamine (PtdE), and the diglyceride 1,2-diolein, on the other hand, were totally inactive in this respect. Very similar results were obtained with profilactin- γ prepared as described recently¹¹. In controls with actin alone and with actin plus phosphatidylinositol bisphosphate (PtdInsP₂), the $t_{1/2}$ of polymerization was 21 and 17 min, respectively.

When profilactin was instead mixed with the phospholipid vesicles at an increased salt concentration, 80 mM KCl in the presence of 0.1 mM CaCl₂, 10 μ M EDTA, 12 μ M EGTA, 0.5 mM ATP, 0.5 mM DTT, 5 mM potassium phosphate buffer pH 7.6 (omitting the preincubation in low salt), both PtdIns(4,5)P₂ and phosphatidylinositol 4-monophosphate (PtdIns(4)P) exhibited high activities, with $t_{1/2}$ values of 16 and 35 min, respectively (Fig. 1), whereas PtdIns from pig liver and PhA gave respective $t_{1/2}$ values of 85 and 90 min; PtdS (and PtdIns from soy bean, not shown) was almost inactive ($t_{1/2} > 150$ min). The finding that PtdIns(4,5)P₂ has no major effect on the polymerization of actin alone, and that the kinetics and extent of actin polymerization from profilactin in its presence

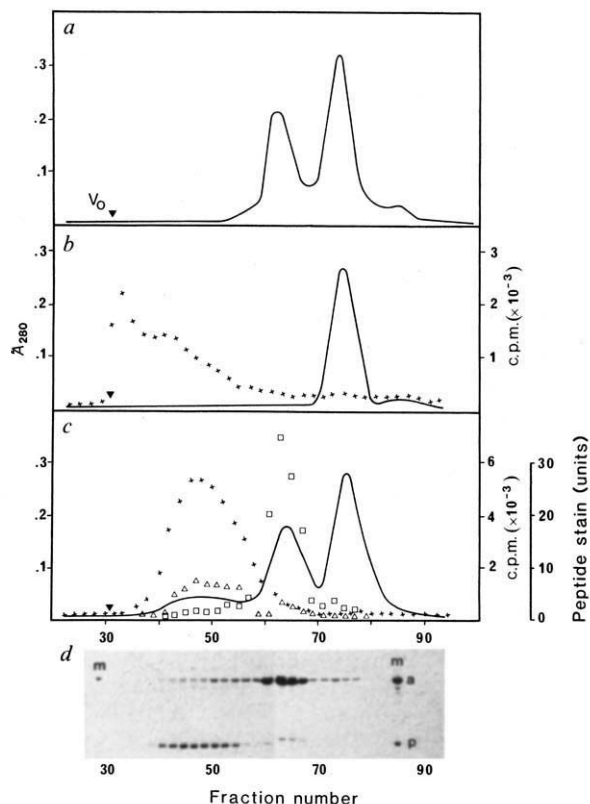
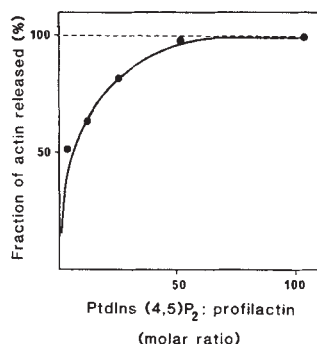


Fig. 2 The effect of PtdIns(4,5)P₂ on the chromatographic behaviour of profilactin. PtdIns(4,5)P₂ (0.8 mg; M_r 1,132)¹³ was mixed with trace amounts of ¹⁴C-labelled PtdIns in chloroform/methanol (4:1 and dried under vacuum. The phospholipids were then sonicated in 0.1 ml of G-buffer under nitrogen, immediately mixed with 0.4 ml of G-buffer containing 0.8 mg of profilactin- β and incubated for 10 min at room temperature, after which the incubation mixture was chromatographed at 4 °C on a column (1 $\times$ 56 cm) of Sephacryl 400 (Pharmacia) equilibrated with G-buffer (c). The fractions (0.5 ml per 2 min) were analysed for absorbance at 280 nm (—), radioactivity ($\times$), profilin (Δ) and actin ($\square$). To determine the relative amounts of profilin and actin, fractions of the chromatogram were analysed by electrophoresis on SDS-15% polyacrylamide gels⁴ (d). The gels were scanned with an LKB 2202 Ultrosan after staining with Coomassie blue. Separate control runs of the same amounts of profilactin and PtdIns(4,5)P₂ are shown in a and b, respectively. In d, m represents marker profilactin, and a and p actin and profilin, respectively.

are closely similar to those of actin alone suggest that PtdIns(4,5)P₂ effectively eliminates the activity of profilin. (Preliminary experiments indicate that inositol trisphosphate has no effect on the stability of profilactin, but counteracts the effect of PtdIns(4,5)P₂. Furthermore, PtdIns(4,5)P₂ in mixed vesicles containing PtdIns(4,5)P₂, PtdC and PtdE in the proportions 10:45:45 is still highly active towards profilactin.)

Figure 2 illustrates the effect of PtdIns(4,5)P₂ on the chromatographic behaviour of profilactin at low salt concentrations and in the presence of 0.1 mM CaCl₂. In aqueous solutions, PtdIns(4,5)P₂ forms micelles of relative molecular mass (M_r) 93,000 (ref. 12). Chromatography on Sephacryl 400 alone produced low recovery of the phospholipid (20%). A large fraction of the eluted phospholipid appeared with the void volume (V_0), the rest being spread out over the first half of the chromatogram (Fig. 2b). When the PtdIns(4,5)P₂/profilactin mixture was analysed, however, a peak containing 50% of the phospholipid was eluted well behind the V_0 . The absorbance profile obtained (Fig. 2c) had three major peaks, one coinciding with the phospholipid, one which was eluted at a position close to that of profilactin, and one which was eluted with the internal volume of the column. The last peak contained the ATP present in the incubation mixture. The distribution of profilin and actin over the chromatogram was determined by analysing selected fractions

Fig. 3 Concentration dependence of the effect of PtdIns(4,5)P₂ on profilactin. Profilactin- β was mixed with PtdIns(4,5)P₂ at different molar ratios in the viscometer, and filament formation was followed as in Fig. 1. The fractions of the profilactin dissociated by the phospholipid were determined from the maximal viscosities reached, compared with that reached after addition of MgCl₂ (2 mM; see Fig. 1 legend).



by electrophoresis and scanning the gels (Fig. 2d). Gel scanning (Fig. 2c) indicated that the major part (88%) of the profilin co-chromatographed with the phospholipid and that 88% of the actin appeared as G-actin in the second peak. All the protein applied to the column was recovered. Thus, PtdIns(4,5)P₂ produced efficient dissociation of the profilactin complex, which apparently resulted from a relatively strong binding of profilin to the PtdIns(4,5)P₂ particles. The monomeric actin released, on the other hand, seemed unaffected by the phospholipid.

As PtdIns(4,5)P₂ clearly exerts its effect by binding to profilin, and as PtdIns(4,5)P₂ seems to have little effect on the polymerization of actin itself, it should be possible to estimate the stoichiometry of the PtdIns(4,5)P₂/profilin interaction indirectly by measuring the fraction of actin released using the viscosimetric assay at limiting amounts of PtdIns(4,5)P₂. For this, the fraction of actin released was determined by recording the maximum viscosity reached at varying concentrations of PtdIns(4,5)P₂. We found a direct relationship between the amount of PtdIns(4,5)P₂ added and the fraction of actin converted to filaments at low concentrations of phospholipid (Fig. 3), and from the initial part of the dose-response curve, calculated that dissociation of the protein complex required ~10 molecules of PtdIns(4,5)P₂ per molecule of profilactin. Considering the geometry of the PtdIns(4,5)P₂ micelles (spherical particles with a Stokes radius of 39 Å and a packing number of 82)¹² and of profilactin (Stokes radius 29 Å), this result indicates the possibility of a specific binding site for PtdIns(4,5)P₂ on profilin.

Clearly, binding of PtdIns(4,5)P₂ to profilactin causes the release of actin which is then available for polymerization in suitable conditions. Thus, the possibility should be considered that this phospholipid is involved in inducing actin filament formation in the cell. It has been realized for some time that ligand-induced turnover of phosphatidylinositol and mobilization of intracellular calcium may be causally related in many systems¹³. It was shown recently that a very early event following such receptor-mediated stimulation is the hydrolysis of PtdIns(4)P and PtdIns(4,5)P₂, resulting in the intracellular release of inositol 1,4-bisphosphate (InsP₂) and inositol 1,4,5-trisphosphate (InsP₃)¹⁴⁻¹⁹. The primary product, InsP₃, in turn seems to act as a second messenger for the induction of Ca²⁺ translocation²⁰⁻²³. Unstimulated cells contain a preponderance of PtdIns over the phosphorylated forms PtdIns(4)P and PtdIns(4,5)P₂, suggesting that the stimulated phosphorylation of PtdIns to PtdIns(4)P and PtdIns(4,5)P₂ through the action of membrane-associated kinase activities may be an equally early event (for review see ref. 24).

It is interesting that the increased activity in the phosphatidylinositol cycle induced by ligand-receptor interactions in turn seems to be closely correlated with an increased formation of microfilaments (polymerization of actin). Direct analysis of the G/F-actin ratio in cell extracts using the DNase I inhibition assay has shown that there is a rapid polymerization of actin following stimulation²⁵⁻³⁰ of, for example, platelets with thrombin or ADP²⁵⁻²⁷ and pancreatic β -cells with glucose²⁸⁻³⁰. In such cases, the ligand-receptor interaction has been shown to be linked to an increased turnover in the PtdIns cycle and Ca²⁺

mobilization³¹⁻³⁶. Furthermore, ultrastructural studies suggest that one of the immediate events following stimulation of serum-starved glia-like cells and fibroblasts by platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) is the polymerization of actin, causing an outgrowth of new membrane lamellae and microspikes⁸; this phase is followed by increased cell motility. Both PDGF and EGF cause increased PtdIns turnover^{37,38}. Finally, similar morphological changes, although less well characterized, have been observed after activation of *src* gene in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus³⁹; it is now known that the *src* gene product can phosphorylate PtdIns(4)P to PtdIns(4,5)P₂, suggesting its involvement in the PtdIns cycle⁴⁰.

The results presented here provide biochemical evidence for a specific effect of PtdIns(4,5)P₂ on the putative precursor of microfilaments, profilactin. This may indicate that the formation of microfilaments and thus cell motility is regulated through the PtdIns cycle.

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1. Tilney, L. G., Bonder, E. M. & DeRosier, D. J. *J. Cell Biol.* **90**, 485-494 (1981).
2. Carlsson, L., Markey, F., Blikstad, I., Pearson, T. J. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6376-6383 (1979).
3. Markey, F., Persson, T. & Lindberg, U. *Cell* **23**, 145-153 (1981).
4. Malm, B., Larsson, H. & Lindberg, U. *J. Muscle Res. Cell Motil.* **4**, 569-588 (1983).
5. Poste, G. & Nicolson, G. L. (eds) *Cytoskeletal Elements and Plasma Membrane Organization* (North-Holland, Amsterdam, 1981).
6. Lindberg, U., Höglund, A.-S. & Karlsson, R. *Biochimie* **63**, 307-323 (1981).
7. Small, J. V. & Langanger, G. *J. Cell Biol.* **91**, 695-705 (1981).
8. Mellström, K. et al. *J. Muscle Res. Cell Motil.* **4**, 589-609 (1983).
9. Markey, F., Larsson, H., Weber, K. & Lindberg, U. *Biochim. biophys. Acta* **704**, 43-51 (1982).
10. Markey, F., Persson, T. & Lindberg, U. *Biochim. biophys. Acta* **709**, 122-133 (1982).
11. Segura, M. & Lindberg, U. *J. Cell. Biol.* **259**, 3949-3954 (1984).
12. Sugiyama, Y. *Biochim. biophys. Acta* **641**, 148-159 (1981).
13. Michell, R. H. *Biochim. biophys. Acta* **415**, 81-147 (1975).
14. Agranoff, B. W., Murthy, P. & Seguin, E. B. *J. Biol. Chem.* **258**, 2076-2078 (1983).
15. Berridge, M. J., Dawson, M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. *Biochem. J.* **212**, 473-482 (1983).
16. Berridge, M. J. *Biochem. J.* **212**, 849-858 (1983).
17. Martin, T. F. J. *J. biol. Chem.* **258**, 14816-14822 (1984).
18. Rebecchi, W. J. & Gershengorn, M. C. *Biochem. J.* **216**, 299-308 (1983).
19. Downes, C. P. & Wusterman, M. M. *Biochem. J.* **216**, 633-640 (1983).
20. Streh, H., Irvine, R. F., Berridge, M. J. & Schultz, I. *Nature* **306**, 67-68 (1984).
21. Burgess, G. et al. *Nature* **309**, 63-66 (1984).
22. Prentki, M. et al. *Nature* **309**, 562-564 (1984).
23. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. Biol. Chem.* **259**, 3077-3081 (1984).
24. Berridge, M. J. *Biotechnology* **2**, 541-546 (1984).
25. Pribluda, V. & Rotman, A. *Biochemistry* **21**, 2825-2832 (1982).
26. Fox, J. E. B. & Phillips, D. R. *Nature* **292**, 650-651 (1981).
27. Casella, J. F., Flanagan, M. D. & Lin, S. *Nature* **293**, 302-305 (1981).
28. Swanson-Flatt, S. K., Carlsson, L. & Gylfe, E. *FEBS Lett.* **117**, 299-302 (1980).
29. Rao, K. M. & Varani, J. *J. Immun.* **129**, 1605-1607 (1982).
30. Laub, F., Kaplan, M. & Gitler, C. *FEBS Lett.* **124**, 35-38 (1981).
31. Rittenhouse-Simmons, S. *J. clin. Invest.* **63**, 580-587 (1979).
32. Bilal, M. M. & Lapetina, E. G. *J. biol. Chem.* **257**, 11856-11859 (1982); *Biochem. biophys. Res. Commun.* **109**, 217-222 (1982).
33. Zawali, W., Brown, C. & Rasmussen, H. *Biochem. biophys. Res. Commun.* **117**, 448-455 (1983).
34. Cockcroft, S., Bennet, J. P. & Gomperts, B. D. *FEBS Lett.* **110**, 115-118 (1980).
35. Volpi, M., Yassin, R., Naccache, P. H. & Shāfi, R. I. *Biochem. biophys. Res. Commun.* **112**, 957-964 (1983).
36. Hasegawa-Sasaki, H. & Sasaki, T. *J. Biochem., Tokyo* **91**, 463-468 (1982).
37. Habenicht, A. J. R. et al. *J. biol. Chem.* **256**, 12329-12335 (1981).
38. Sawyer, S. T. & Cohen, S. *Biochemistry* **20**, 6280-6286 (1981).
39. Boschek, C. B. et al. *Cell* **24**, 175-184 (1981).
40. Sugimoto, Y., Whitman, M., Cantley, L. C. & Eriksson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2117-2121 (1984).

Erratum

Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor

T. Shirai, H. Yamaguchi, H. Ito, C. W. Todd & R. B. Wallace
Nature **313**, 803-806 (1985)

ON page 805, Figures 2 and 3 were transposed.

The duplex brain

John C. Marshall

Cerebral Dominance: The Biological Foundations.

Edited by Norman Geschwind and Albert M. Galaburda.
Harvard University Press: 1984. Pp.232. \$27.50, £27.50.

CEREBRAL dominance, the specialization of the left and right sides of the brain for different functions, was *almost* discovered some two millennia ago. The physicians who compiled the Hippocratic Corpus observed that injury to one side of the head was frequently associated with paralysis of the opposite side of the body; they further noted that loss of language was usually associated with *right* hemiparesis. For us, the conclusion that the left hemisphere is dominant for language processing seems inescapable. Yet neither the Greek doctors nor the many later European neurologists who made similar observations did draw this conclusion. It was not until the 1860s that Paul Broca convinced the scientific community that "On parle avec l'hémisphère gauche". From La Salpêtrière in Paris, Broca reported a series of autopsied cases of aphasia with relatively focal left-hemisphere lesions and argued that the dominance of the left half of the brain was expressed in its control of both language and handedness.

Two questions therefore arose. How are the brains of left-handed people organized? And what does the right (non-dominant) hemisphere do? Broca thought that the left-handed brain was simply the mirror image of the "normal" brain. We now know that this is not the case; most left-handers have left hemisphere dominance for language. Many nineteenth-century neurologists also seem to have believed that the right hemisphere of right-handers was merely a spare, functionless entity that only came into use by "taking over" the functions of the left, should that hemisphere be damaged. This claim likewise proved false when, in 1876, the English neurologist John Hughlings Jackson showed that the right hemisphere played the leading role in "visual ideation". Thus arose the modern notion of "complementary specialization".

Over the succeeding century a vast body of work has correlated particular functional deficits with damage to more or less precisely defined regions of the brain. Yet the question of *why*, for example, the left hemisphere is (usually) dominant for language was seldom broached. In the past 15 years, however, a small group of investigators has mounted a vigorous effort to discover the biological foundations of complementary specialization in the duplex brain. The volume under review presents a somewhat manic survey of this work, a survey in which eagerness sometimes outstrips the data, loose ends dangle precariously and counter-evidence quietly dis-

appears. Yet if even half the ideas expressed are true, our understanding of cerebral dominance will have deepened very considerably. And if some of them can be translated into clinical practice the prospects for recovery from disabling brain damage will be immeasurably improved.

First, then, some facts. It is now beyond dispute that many adult human brains show gross anatomical asymmetries that

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The brain exposed — wood cut from De Humani Corporis Fabrica by A. Vesalius (1543).

reflect the size of specialized cytoarchitectonic areas (discussed in the book by Geschwind and Galaburda); some of these asymmetries can be observed *in vivo* by radiological techniques, some are present in infants and fetuses, and some have even left their mark in the fossil record as far back as Peking man (LeMay). More subtle asymmetries, the differential branching of dendritic trees, for example, may also exist (Scheibel). Many lateral asymmetries in the concentration of neurotransmitters and in susceptibility to pharmacological agents are likewise coming to light (Galaburda).

Do these asymmetries have any functional significance or are they simply random variation? That the typical direction of the asymmetry often corresponds with known behavioural specialization certainly predisposes one to the former view. Thus lesions to the left planum temporale are a

common cause of Wernicke's aphasia, and this area is usually larger on the left. Direct evidence would, however, be more convincing. Sometimes that evidence is positive: Ratcliff *et al.* (*Brain and Language* 11, 87-98; 1980) found lateral asymmetries of the posterior Sylvian branches of the middle cerebral artery that predicted language dominance as assessed by successive pharmacological inactivation of each hemisphere. Sometimes it is not: Henderson *et al.* (*Neurology* 34, 1,086-1,089; 1984) failed to find a correlation between anatomical asymmetries of the language areas (as assessed by computed X-ray tomography) and known language dominance in two groups of patients who had, respectively, sustained aphasia after left-brain damage (the typical case) and after right-brain damage (the atypical case).

In one sense, current research suffers from an *embarras de richesses*. As more and more physical asymmetries are uncovered, the task of discovering which of them have genuine psychological significance becomes progressively harder. This issue is inadvertently highlighted in chapters by Kemper and by Duffy, MacAnulty and Schachter. Kemper and Galaburda have found multiple congenital lesions in the brains of patients with developmental dyslexia; Duffy *et al.* have found widespread electrophysiological abnormalities in the same population. The latter authors are thus led to propose that "Dyslexia pure may represent dysfunction within the entire widely distributed brain system devoted to language processing". But if this is so, we are left with the mystery of why the behavioural deficits are so specific to reading and spelling.

Animal models of cerebral dominance would no doubt help distinguish causal from merely correlational phenomena: controlled experiments with animals can supplement the study of fortuitous lesions in man. Although animals do not talk (with either hemisphere), convincing evidence of both functional and physical asymmetries is now accumulating in many non-human species.

In the rat, for example, not only are there hemispheric differences in cortical thickness (Diamond), but there is also lateralization of neuroendocrine control of grooming (Gerendai); lateralization of circling behaviour consequent upon differences in dopamine concentration in the two striata (Glick and Shapiro); and lateralization of the mechanisms involved in spatial choice, taste aversion and muricide (Denneberg). Denneberg's chapter is outstanding for the clarity with which it outlines how simple models of the logic of activation and inhibition within and between the hemispheres can be used to interpret the results of lesion experiments.

One area where animal models will play an important role concerns the effects of testosterone on the developing brain. Geschwind and Behan argue that excess

testosterone slows the rate of growth of the left hemisphere and suppresses the development of the thymus gland. They thereby attempt a unified explanation of the apparent over-representation of males and left-handers who present with developmental learning disorders and certain auto-immune diseases. This left hemisphere retardation may alter the brain's pattern of connectivity as competition for synaptic space takes place in the developing cortical plate, a process for which Goldman-Rakic and Rakic have provided an elegant experimental analysis in monkey. Nonetheless, one would like to see Geschwind and Behan's epidemiological observations replicated by independent investigators. In the past, left-handers have proved a notoriously unreliable group with respect to almost any measure of neuropsychological functioning one can think of. Curiously, Geschwind and Behan fail to mention the traditional story that left-handers are, on average, poor at visuo-spatial skills, for which right-hemisphere pathology would be implicated; they also ignore Kimura's recent evidence (*Human Neurobiology* 2, 147-154; 1983) that left-handers, as a group, are just as left-dominant for speech representation as right-handers.

The best animal analogue of human vocal learning is to be found in bird-song. Song learning in canaries is, like human speech, "a left-sided affair" (Nottebohm). Lesion of the left hypoglossus nerve, which controls the left half of the syrinx, has a far more deleterious effect upon singing than does lesion of the right. More surprisingly, seasonal variation in the pattern of song appears to be determined by the growth of new neurones and synapses. Although there is currently no evidence for such synapto- and neuro-genesis in the adult human brain, Nottebohm speculates that:

In the not-so-distant future it may be possible to treat local regions of the brain to activate genes that induce dendritic retraction and growth, that induce synaptic formation and shedding, that induce birth, migration, and differentiation of new neurones.

At present, Nottebohm's "hope for a new neurology" is science-fiction although the advances of the past decade do raise the serious possibility that the diagnosis of "irreversible" brain damage will eventually give way to a less horrendous prognosis. Such success would be a fitting tribute to Professor Geschwind, who died at a tragically early age last year. The emerging synthesis that this book displays can in large part be credited to the enthusiasm, dedication and insight with which Norman Geschwind always insisted that cerebral dominance does have demonstrable biological foundations. □

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All keyed up in the laboratory

E.G. Richards

Computers in Biology: An Introduction.

By Robert Ransom and Raymond J. Matela.

Open University Press, Milton Keynes/
Taylor & Francis, Philadelphia: 1985.
Pp.150. £9.95, \$19.

Microcomputers in Biology: A Practical Approach.

Edited by C.R. Ireland and S.P. Long.
IRL: 1984. Pp.324. Pbk £15, \$27.

Mathematics, Biology and Microcomputers.

By A.N. Barrett.

Elsevier-Biosoft: 1984. Pp.102. £24, \$36.

THE day has not quite arrived when a biologist, unaccustomed to computers, can use one as he might use a centrifuge. He must go through the laborious and daunting process of coming to terms with whatever computer is available to him, its jargon, its hardware and its capabilities, its operating system and more besides. Having done that he then must either set about learning its language and write a program for the task he has in mind (and in the process more than likely reinvent the wheel) or, if he is lucky, use software written by somebody else.

Even then he is likely to encounter many pitfalls: the program may require modification before it will run on his computer (even if it is written in a language familiar to his machine); the documentation may be inadequate or even absent; it may lack robustness and die on him with an obscure error message as a result of a mis-key after he has been entering data for the last hour; he may be lulled into a false sense of security by a program which presents him with the unadorned slope of a straight line fitted to data points which are scattered at random.

Help in coming to terms with all this is provided by Ransom and Matela in *Computers in Biology*, a short but comprehensible account of some aspects of computer science. The book first explains how computers might be useful and what is involved in using them. It goes on to introduce data structures, lists and trees and the like, and then to discuss statistical analyses using computers, computer graphics, interfacing experimental apparatus with computers and finally the simulation of models for biological processes. One appendix is used to describe briefly computer hardware as we know it today, and discuss how to choose a computer (including a section on how to deal with computer salespeople), while another introduces operating systems and the facilities they might offer. A useful glossary is also provided.

In a book of such generality, no program

listings are included but the text is illustrated with many examples of how computers might be applied in various branches of biology. Even if it does not cover your pet subject, it may give you seminal ideas.

Not every research worker has access to a mainframe or a mini, and may have to contemplate choosing a microcomputer. Assistance in this confusing task is at hand in *Microcomputers in Biology*, a multi-authored work edited by Ireland and Long. The first chapter goes some way to explain some of the jargon, also offering advice on choosing a microcomputer, and the book continues with chapters on the mysteries of interfaces, the problems involved in data-logging and the possibilities inherent in computer graphics. These are useful epitomes for workers with some experience in computing but may present problems to the complete beginner. The book includes summaries of some ways in which microcomputers can be used to control apparatus or analyse data obtained from several analytical techniques: spectrophotometry, chromatography and ultracentrifugation, among them. It ends with a chapter on controlling environmental factors. There is also a chapter on doing various things with nucleic acid sequences, which, it should be noted, does not attempt to come to terms with using sequence databases (such as is available from EMBL) with microcomputers.

The book should appeal to biologists who use such techniques and have some flair for instrumentation, and who are contemplating using a microcomputer. Most chapters end with listings of programs, usually in BASIC, which run on the author's microcomputer (discs containing versions of these programs are also to be available for four popular microcomputers). It is unclear how easy these will be to use, how robust they are or to what extent they protect the user from drawing false conclusions.

Barrett's *Mathematics, Biology and Microcomputers* also provides listings of simple programs (for instance to solve quadratic equations, though many of them would be better tackled on a hand calculator) but most of his book is taken up with an introduction to BASIC, solving quadratic and cubic equations (but stopping short of iterative methods for solving general equations), matrices, coordinate geometry, curve fitting, simple mensuration and so on. It is essentially an introduction to certain parts of mathematics and as such reflects, as do the biological examples, what are presumably the author's interests. It ends with a somewhat mysterious section on the manipulation of biological data by prime numbers. Unfortunately the reference to further details of this is still in press. □

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Making good

Alan R. Lehmann

DNA Repair.

By Errol C. Friedberg.

W.H. Freeman: 1985. Pp.614. \$39.95, £28.95.

WORK on oncogenes over the past three years has shown conclusively that a crucial step in the conversion of a normal cell to a cancer cell involves a specific alteration in the cellular DNA, whether this be a single base change or a chromosome rearrangement. In many (if not all) cancers, the initiating event is known to be the interaction of a carcinogenic agent with cellular DNA to produce some form of alteration or damage to the DNA. All cells have evolved a complex series of enzymatic repair pathways to protect themselves from the potentially devastating effects of such damage. The importance of these pathways is shown in a number of human genetic disorders (of which xeroderma pigmentosum is the best known example), in which a molecular defect in a DNA repair pathway may lead not only to an increased frequency of carcinogenesis, but also to disorders in the neurological, immune and vascular systems.

DNA Repair provides a comprehensive, detailed and up-to-date description of current knowledge of this subject. The format is similar to *DNA Replication* by Arthur Kornberg, and like that book Friedberg's account is likely to become a classic treatise for postgraduates and research workers.

The subject is covered in a logical manner, starting with the different types of damage produced in DNA by various carcinogens, and simple enzymatic reversal mechanisms. Early events in excision repair are covered with the emphasis on detailed enzymatic mechanisms. Following chapters deal with later steps in excision repair, and with the tolerance mechanisms whereby cells are able to cope with damage which is not removed from their DNA. In each section the relatively well characterized mechanisms elucidated in bacteria are described first, followed by the inevitably more poorly understood processes in eukaryotes. The discussions of the complicated and controversial topics dealt with in the later part of the book are clear and balanced, Friedberg taking especial care to distinguish between fact and speculation.

My only criticism of the book concerns a part of Chapter 1. Here the reader is thrown headlong into some rather subtle points to do with errors in DNA replication with insufficient introduction and definition of terms, and the section would have been better placed in a later chapter. This, however, is merely a niggle. One of the many virtues of *DNA Repair* is the depth and breadth of the literature citations, each of the nine chapters being accompanied by about 200 references; I was unable to find a single important reference which had been omitted. The diagrams are clear and uncluttered, the index is comprehensive and altogether it is difficult to see how the book could have been improved. □

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first two (Hippocrates and Euclid) 59. There are a number of small factual slips for detection by the knowledgeable, and some questionable judgements: to hold that the Hippocratic corpus is "arguably the oldest western scientific text" is very much a matter of definition, for example. But preponderantly the book conveys sound information and sensible opinions about what are, by any standards, works of outstanding importance in the history of

PHILOSOPHIÆ NATURALIS PRINCIPIA MATHEMATICA

Auctore JS. NEWTON, Trin. Coll. Cantab. Soc. Matheseos
Professore Lucasiano, & Societatis Regalis Sodali.

IMPRIMATUR.
S. PEPYS, Reg. Soc. PRÆSES.
Julii 5. 1686.

LONDINI,
Jussu Societatis Regiæ ac Typis Josephi Streater. Prostant Ven-
terapud Sam. Smith ad insignia Principis Wallie in Cœmiterio
D. Pauli, alioq; nonnullis Bibliopolas. Anno MDCLXXXVII.

Classic beginning — the title page of Newton's *Principia*.

science. The format is fairly regularly maintained: author's career, context of the book, its content, its reception and its publishing history.

One might describe these twelve vignettes as basic history, and recognize that taken together they give an odd and partial view of the development of science. Presumably it was not the author's intention that a reader should form such a distorted synoptic view by reading steadily through from page 1 to page 357, but rather that he would read particular sections according to his interests and needs. Treated as a mini-encyclopaedia this is a very useful book, capable of stimulating as well as informing. But there are of course other worlds in the history of science to which this method of approach and this choice of examples give no entrance: the danger of Mr Gjertsen's book might be in concealing the existence of such worlds and the merit of exploring them. Again, he probably did not intend to use the Kuhnian language of "scientific revolutions" to recommend a "great men" account of history since his short studies often draw attention to the force of parallelism and convergence (Darwin-Wallace, for example). But in the last resort the continuity of the past — continuity so obvious to us in our own present — is its feature that most resists historical reconstruction, and cannot be promoted by a Horner-like picking out of the plums, even dried plums.

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Master theses

A. Rupert Hall

The Classics of Science: A Study of Twelve Enduring Scientific Works.

By Derek Gjertsen.

Lilian Barber Press, New York and
Gravesend: 1984. Pp.374. Hbk \$24.95,
£20; pbk \$15.95, £12.95.

WHAT is a scientific classic? We may all recognize the type without agreeing upon the supreme examples of it; a classic is on a pedestal. No matter that it is rarely read, and never for pleasure, it is universally admired.

Most with any claim to an opinion would include among the great scientific writers Aristotle and Archimedes (both omitted from this selection), Harvey, Newton, Darwin . . . but there comes a point when the selection of Dalton to the exclusion of Lavoisier (as in the present volume) becomes a mere matter of taste. One could certainly publish a volume parallel to Mr Gjertsen's where a different selection would possess equal scientific merit and in-

clude areas (for example cytology and particle physics) completely neglected by him. Perhaps it is natural that a British author and an American publisher should combine to display five out of twelve scientific classics written by Englishmen; indeed, after Galileo all the "classic" scientists are English with the unique exception of Linnaeus!

The conception of a "classic", therefore, if restricted to twelve or twenty or even a hundred seems devoid of precise meaning. To say that is not to judge Mr Gjertsen's treatment of twelve familiar works from the *Corpus Hippocraticum* to the *Origin of Species* as without value or interest. He has done his work well and each short study is a useful introduction to a specialist literature as well as a review of modern interpretations and critical assessments. Not everyone knows that Lyell published roughly a volume for each year of his writing life, or that the *Origin* has been translated into Latvian (besides 28 other languages). Some "classics" are better and more elaborately treated than others: the last two of them — hardly poles apart in any sense — Lyell's *Principles* and Darwin's *Origin* together rate 66 pages; the

How do enzymes work?

M.I. Page

The Bioorganic Chemistry of Enzymatic Catalysis.

By Myron L. Bender, Raymond J. Bergeron and Makoto Komiyama.
Wiley: 1984. Pp.312. £50.05, \$39.50.

IT IS fashionable to justify the publication of work on some aspects of chemistry by invoking its relevance to the phenomena of life. Often this is unnecessary, because the chemistry is of interest in its own right (while sometimes the claim is exaggerated). In particular the no-man's-land between traditional disciplines is always exciting because this is where fresh understanding often emerges. The pioneering research of Myron Bender is a case in point; Bender helped to bridge the gap between chemistry and biochemistry and to define a new borderline area. Today the application of the techniques and phraseology of physical organic chemistry to enzyme catalysis is accepted practice and owes much to his work.

Our response to fundamental questions of life is usually to resort to models we think we understand. By definition, the model is a simpler system and only reflects a small part of the observed behaviour of the living system. Ill-defined separation of causes and effects *in vivo* may lead to only partial answers.

The fullness of an answer is of course determined by the question — in this case, how do enzymes work? In their book, Bender, Bergeron and Komiyama have

aimed to show how an understanding of organic reaction mechanisms is relevant to an understanding of how enzymes work. They accept and propagate the view that the mechanisms — that is, the routes of bond making and breaking processes — of reactions are similar in non-enzyme and in enzyme-catalysed reactions. This is almost certainly correct. However, the number of amino acid residues of the enzyme directly involved in bond making and breaking is only a tiny percentage of the total number in the whole enzyme. What is the function of the rest of the enzyme's structure? Why can't the one or two amino acid residues involved in the "mechanism" catalyse the reaction as free amino acids? Besides enhancing the rate of reactions, enzymes are generally very discriminative in their choice of substrates. Why can't an enzyme catalyse the reaction of a compound which contains the necessary reactive function but is smaller than the normal substrate? Questions such as these are not addressed in the book.

It is not obvious that three authors have been involved in the writing, and the style throughout is uniform. This is an authoritatively written and well illustrated account of physical organic chemistry which should be useful to advanced undergraduate and graduate students wanting an understanding of the mechanism of reactions and an answer to why and how catalysis occurs. It is at its best when simple chemical reactions are discussed, but sadly fails to hint at the unsolved or controversial aspects of enzymatic catalysis. □

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Viruses in disease

Clive Sweet

Concepts in Viral Pathogenesis.

Edited by Abner Louis Notkins and Michael B.A. Oldstone.
Springer-Verlag: 1984. Pp.409. \$34.40, DM 94.

Viral Pathogenesis and Immunology.

By Cedric A. Mims and David O. White.
Blackwell Scientific: 1984. Pp.398. Pbk £14.80, \$26.

UNTIL recently, most virologists have shied away from studies of pathogenesis, regarding them as too difficult, less interesting or less satisfactory than the more molecular aspects of virology. Much attention has been given to the structure and biochemistry of viruses, and mechanisms of replication in cell culture, but virus pathogenicity — that is, the ways in which they produce disease — has been relatively neglected. This emphasis is changing and there is now a keen interest in the mechan-

isms of disease production, possibly originating from a clearer understanding of host defence mechanisms but given impetus by the merging of interests of molecular virologists and those more concerned with the causation of disease. Pioneering work with segmented RNA viruses (reoviruses and orthomyxoviruses) has led to identification of virus genes and their products and has resulted in a better understanding of virus virulence at the genetic and molecular level. The new area of recombinant DNA technology has allowed these approaches to be extended to non-segmented RNA (polio) as well as DNA (herpes simplex, pseudorabies) viruses.

While studies on virus pathogenicity hold great promise, they demand a breadth of understanding across many specialized areas. Knowledge of virus structure, genetics and replication, together with broader aspects of cell and host biology, is required for a full appreciation of the subject. Consequently, the writing of a textbook on the subject is a daunting task. The two books reviewed here have tackled this problem in different ways.

The editors of *Concepts in Viral Pathogenesis* have chosen the multiauthor approach. No fewer than 75 authors have contributed a total of 53 articles under the headings of "Host Control of Viral Spread", "Host Range and Tissue Tropism", "Virus Maturation and Diversity", "Virus Persistence", "Virus-interaction with the Immune System", "Evolving Concepts in Viral Pathogenesis" and finally "Control of Viral Disease". The book is intended to be a series of mini-reviews and editorials, 1,000–2,000 words in length, each a "pithy distillation of the state-of-the-art".

Sadly, the book does not attain these objectives. Although all of the articles contain much information they often amount only to thumbnail sketches and are too short to be really useful. However, they are generally well referenced (with one notable exception where no references are given), allowing easy access to more detailed information. Inevitably, there is some duplication between chapters, (fortunately never contradictory), and some areas are ignored. For example, there is no chapter on virus-dependent damage (cytotoxicity) and the impression gained from reading the book is that viruses do not in themselves cause disease, this being almost entirely due to aberrations in host responses. A consideration in relevant chapters of how viruses avoid, counteract or fail to induce host responses would also have been useful. Nevertheless, the book is a helpful source of reference and concepts. Typographical errors are few but the occasional use of abbreviations without definition is annoying.

In contrast Mims and White have produced an excellent text with just two contributors. Their book is packed full of information in an easily digestible, interesting and entertaining style. It covers in considerable detail the various aspects of virus pathogenesis — basic characteristics of viruses, infection and spread of viruses through the body, the immune response to viral infection, mechanisms of disease production, determinants of viral virulence and host resistance, persistent infections and finally immunization against virus diseases — from the point of view of both the host and the pathogen.

The book is especially strong in its treatment of the immune response. Indeed, the depth of coverage of all topics makes it suitable not only for medical and science undergraduates and postgraduates but also for scientists in, or moving into, viral pathogenesis research, for whom the up-to-date and extensive reference section should prove especially valuable. Clear diagrams and tables illustrate many points throughout the book and each chapter concludes with a summary and selected readings. □

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Earth Sciences

Aquatic and Terrestrial Humic Materials. R.F. CHRISTMAN and E.T. GJESSING (eds). *Ann Arbor: 1983. Pp. 538. ISBN 0-250-40550-4. £37.*

Future Weather: Carbon Dioxide, Climate and the Greenhouse Effect. J. GRIBBIN. *Pelican: 1983. Pp. 272. Pbk ISBN 0-14-022459-9. £3.50.*

Numerical Prediction and Dynamic Meteorology, 2nd Edn. G.J. HALTINER and R.T. WILLIAMS. *Wiley: 1983. Pp. 477. ISBN 0-471-05971-4. Np.*

Red Sky at Night. By JOHN BARRINGTON. *Michael Joseph: 1984. Pp. 208. ISBN 0-7181-1808-1. £8.95.*

Biological Sciences

The Growth and Development of Birds. By RAYMOND J. O'CONNOR. *Wiley: 1984. Pp. 315. ISBN 0-471-90345-0. £20.*

Handbook of Vitamins: Nutritional, Biochemical, and Clinical Aspects. LAWRENCE J. MACHLIN (ed.). *Dekker: 1984. Pp. 614. ISBN 0-8247-7051-X. \$95.25, SwFr. 211.*

The Herons Handbook. By JAMES HANCOCK and JAMES KUSHLAN. *Croom Helm: 1984. Pp. 288. ISBN 0-7099-3716-4. £16.95.*

Heterotrophic Activity in the Sea. JOHN E. HOBBIE and PETER J. leB. WILLIAMS (eds). *Plenum: 1984. Pp. 569. ISBN 0-306-41724-3. \$85.*

Insect Ecology, 2nd Edn. By PETER W. PRICE. *Wiley: 1984. Pp. 607. ISBN 0-471-07892-1. £38.40.*

Insects of the Falkland Islands. By GADEN S. ROBINSON. *British Museum (Natural History): 1984. Pp. 38. Pbk ISBN 0-565-00955-9. Pbk £3.50.*

Insects of the World. By ANTHONY WOOTTON. *Facts on File: 1984. Pp. 224. ISBN 0-87196-991-2. \$17.95.*

Introduction to Sensation/Perception, 2nd Edn. By D. H. McBURNEY and V. B. COLLINGS. *Prentice/Hall: 1984. Pp. 385. ISBN 0-13-496019-X. £27.75.*

The Lichen-Forming Fungi. D.L. HAWKSWORTH and D.J. HILL (eds). *Blackie: 1984. Pp. 158. ISBN 0-216-91633-X. Hbk £16.95; pbk £7.95.*

Methods of Protein Analysis. ISTVAN KERESZ (ed.). *Halsted: 1984. Pp. 371. ISBN 0-470-27497-2. £39.50.*

Milkweed Butterflies. By P.R. ACKERY and R.I. VAN-WRIGHT. *British Museum (Natural History): 1984. Pp. 425. ISBN 0-565-00893-5. Np.*

The Molecular Biology of Adenoviruses 2. Current Topics in Microbiology and Immunology, Vol. 110. WALTER DOERFLER (ed.). *Springer-Verlag: 1984. Pp. 265. ISBN 3-540-13127-2/0-387-13127-2. DM 132, \$49.50.*

Neurology and Neurobiology, Vol. 11A. The Neurobiology of Zinc, Part A: Physicochemistry, Anatomy and Techniques. C.J. FREDERICKSON, G.A. HOWELL and E. J. KASARSKIS (eds). *Alan R. Liss: 1984. Pp. 404. ISBN 0-8451-2712-8. £44.00.*

Neurology and Neurobiology, Vol. 11B. The Neurobiology of Zinc, Part B: Deficiency, Toxicity and Pathology. C.J. FREDERICKSON, G.A. HOWELL and E. J. KASARSKIS (eds). *Alan R. Liss: 1984. Pp. 360. ISBN 0-8451-2713-6. £41.00.*

Pathology of Skeletal Muscle. By STIRLING CARPENTER and GEORGE KARPATI. *Churchill Livingstone: 1984. Pp. 754. ISBN 0-443-08068-2. Np.*

Patterns of Human Heredity: An Introduction to Human Genetics. By JAMES R. BRENNAN. *Prentice-Hall: 1985. Pp. 340. ISBN 0-13-654245-X. Np.*

Photosynthesis. Vol. II Development, Carbon Metabolism, and Plant Productivity. GOVINDJEE (ed.). *Academic: 1983. Pp. 580. ISBN 0-12-294302-3. \$59.*

Physicochemical Biology Reviews, Vol. 4. V.P. SKULACHEV (ed.). *Harwood: 1984. Pp. 334. ISBN 3-718-0140-0. \$89.50.*

Physiological Correlates of Human Behaviour, Vol. 1 Basic Issues. ANTHONY GALE and JOHN A. EDWARDS (eds). *Academic: 1983. Pp. 350. ISBN 0-12-273901-9. \$39.50.*

The Physiology of Trematodes, 2nd edn. By J.D. SMYTH and D.W. HALTON. *Cambridge University Press: 1983. Pp. 446. Hbk ISBN 0-521-22283-4; pbk ISBN 0-521-29434-7. Hbk £30.00; pbk £11.95.*

Plant Biosystematics. WILLIAM F. GRANT (ed.). *Academic: 1984. Pp. 674. ISBN 0-12-295680-X. \$49.50, £35.*

The Preservation and Maintenance of Living Fungi. By D. SMITH and AGNES H.S. ONIONS. *Commonwealth Mycological Institute: 1983. Pp. 51. Pbk ISBN 0-85198-524-6. Pbk £6.50, \$12.20.*

Proteinase Action. P. ELODI (ed.). *Akademiai Kiado, Budapest: 1984. Pp. 475. ISBN 963-05-3887-3. £28.25.*

Recognition Proteins, Receptors, and Probes: Invertebrates. ELIAS COHEN (ed.). *Alan R. Liss: 1984. Pp. 228. ISBN 0-8451-5007-3. £29.*

Reproduction in Whales, Dolphins and Porpoises. W.F. PERRIN, R.L. BROWNELL and D.P. DeMASTER (eds). *International Whaling Commission: 1984. Pp. 495. ISBN 0-906975-07-7. Np.*

The Role of Extracellular Matrix in Development. 42nd Symposium of the Society for Development Biology. R.L. TRELSTAD (ed.). *Alan R. Liss: 1984. Pp. 662. ISBN 0-8451-1503-0. £58.00.*

Safari: The East African Diaries of a Wildlife Photographer. By GUNTER ZIESLER and ANGELIKA HOFER. *Facts on File: 1984. Pp. 197. ISBN 0-87196-847-9. \$24.95.*

Secretion: Mechanisms and Control. R.M. CASE, J.M. LINGARD and J.A. YOUNG (eds). *Manchester University Press: 1984. Pp. 313. ISBN 0-7190-0975-8. £25.*

Sensory Receptor Mechanisms. W. HAMMAN and A. IGGO (eds). *Wiley: 1984. Pp. 319. ISBN 9971-950-82-0. £52.90.*

Skeletal Research: An Experimental Approach, Vol. 2. ARTHUR S. KUNIN and DAVID J. SIMMONS (eds). *Academic: 1983. Pp. 356. ISBN 0-12-429002-7. \$57.*

Sketchbook of Birds. By C.F. TUNNICLIFFE. *Gollancz: 1984. Pp. 123. Pbk ISBN 0-575-03525-0. Pbk £5.95.*

Spiders of the World. By ROD and KEN PRESTON-MAFHAM. *Facts on File: 1984. Pp. 191. ISBN 0-87196-996-3. \$17.95.*

Staphylococci and Staphylococcal Infections, Vol. 2 The Organisms *In Vivo* and *In Vitro*. C.S.F. EASMON and C. ADLAM (eds). *Academic: 1984. Pp. 827. ISBN 0-12-228102-0. \$70.*

Stratification on Tropical Forests as Seen in Leaf Structure. By I. ROTH. *Dr W. Junk: 1984. Pp. 532. ISBN 90-6193-946-1. \$115, £54.*

Stress, Immunity and Aging. EDWIN L. COOPER (ed.). *Dekker: 1984. Pp. 336. ISBN 0-8247-7114-1. SwFr. 166.*

Striga: Biology and Control. E.S. AYENSU *et al.* (eds). *International Council of Scientific Unions: 1984. Pp. 216. Pbk ISBN 0-930357-01-9. Pbk \$25.*

The Surface-Contact Glia. By FERENC HAJÓS and EDUARDO BASCÓ. *Springer-Verlag: 1984. Pp. 81. Pbk ISBN 3-540-13243-0/0-387-13243-0. Pbk DM 52, \$19.40.*

Themes in Biogeography. J.A. TAYLOR (ed.). *Croom Helm: 1984. Pp. 404. Hbk ISBN 0-7099-2428-3; pbk ISBN 0-7099-2428-3. Hbk £25; pbk £12.95.*

Vicia faba: Agronomy, Physiology and Breeding. P.D. HEBBLETHWAITE *et al.* (eds). *Martinus Nijhoff: 1984. Pp. 333. ISBN 90-247-2964-5. \$53.50, £33.75.*

Vitamins and Hormones, Vol. 40. DONALD B. McCORMICK (ed.). *Academic: 1983. Pp. 312. ISBN 0-12-709840-2. \$39.50.*

Applied Biological Sciences

Advances in Applied Microbiology, Vol. 29. ALLEN I. LASKIN (ed.). *Academic: 1983. Pp. 282. ISBN 0-12-002629-5. \$45.*

Advances in Cancer Control: Epidemiology and Research. PAUL F. ENGSTROM, PAUL N. ANDERSON and LEE E. MORTENSON (eds). *Alan R. Liss: 1984. Pp. 544. ISBN 0-8451-5006-5. £44.*

Advances in Neurology, Vol. 4. R.C. DUVOISIN and A. PLAITAKIS (eds). *Raven: 1984. Pp. 286. ISBN 0-89004-958-0. \$53.50.*

AIDS: A Basic Guide for Clinicians. PETER EBBESEN, ROBERT J. BIGGAR and MADS MELBYE (eds). *Munksgaard: 1984. Pp. 313. ISBN 87-1609715-7. \$40, £29.*

Analgesics: Neurochemical, Behavioral, and Clinical Perspectives. MICHAEL J. KUCHAR and GAVRIL W. PASTERNAK (eds). *Raven: 1984. Pp. 351. ISBN 0-89004-793-6. \$97.50.*

Cell Separation. Methods and Selected Applications, Vol. 3. THOMAS G. PRETLOW II and THERESA P. PRETLOW (eds). *Academic: 1984. Pp. 317. ISBN 0-12-564503-1. \$47.50, £33.50.*

Central and Peripheral Endorphins: Basic and Clinical Aspects. EUGENIO E. MULLER and ANDREA R. GENAZZANI (eds). *Raven: 1984. Pp. 389. ISBN 0-88167-016-2. \$77.*

Computers in Endocrinology. DAVID RODBARD and GIANNI FORTI (eds). *Raven: 1984. Pp. 361. ISBN 0-09004-368-X. \$70.50.*

Progress in Industrial Microbiology, Vol. 19. M.E. BUSHELL (ed.). *Elsevier: 1984. Pp. 466. ISBN 0-444-42364-8. \$88.50, Dfl. 230.*

Protoplast Fusion: Genetic Engineering in Higher Plants. By Y. Y. GLEBA and K.M. SYTNIK. *Springer-Verlag: 1984. Pp. 220. ISBN 3-540-13284-8/0-387-13284-8. DM 148, \$53.90.*

Recent Developments in Clinical Immunology. By W.G. REEVES. *Elsevier: 1984. Pp. 210. ISBN 0-444-80162-6. \$61.50, Dfl. 160.*

Reproduction: The New Frontier in Occupational and Environmental Health Research. JAMES E. LOCKEY, GRACE K. LEMASTERS and WILLIAM R. KEYE Jr (eds). *Alan R. Liss: 1984. Pp. 628. ISBN 0-8451-5010-3. £52.*

Stress-Induced Analgesia. M.D. TRICKLEBANK and G. CURZON (eds). *Wiley: 1984. Pp. 194. ISBN 0-471-90430-9. £19.50.*

Surgical Pathology of the Mediastinum. By ALBERTO M. MARCHEVSKY and MAMORU KANEKO. *Raven: 1984. Pp. 292. ISBN 0-88167-005-7. \$63.*

Viral Pathogenesis and Immunology. By CEDRIC A. MIMS and DAVID O. WHITE. *Blackwell Scientific: 1984. Pp. 398. Pbk ISBN 0-632-01193-9. Pbk £14.80.*

General

Abortion: Understanding Differences. SIDNEY CALLAHAN and DANIEL CALLAHAN (eds). *Plenum: 1984. Pp. 338. ISBN 0-306-41640-9. \$35.*

Advances in Librarianship, Vol. 13. WESLEY SIMONTON (ed.). *Academic: 1984. Pp. 264. ISBN 0-12-02461309. \$32, £22.50.*

Advances in X-Ray Analysis, Vol. 27. JEROME B. COHEN *et al.* (eds). *Plenum: 1984. Pp. 579. ISBN 0-306-41712-X. Np.*

Advancing Agricultural Production in Africa. D.L. HAWKSWORTH (ed.). *Commonwealth Agricultural Bureaux: 1984. Pp. 454. ISBN 0-85198-537-8. £60, \$120.*

Analysis of Foods and Beverages. GEORGE CHARALAMBOUS (ed.). *Academic: 1984. Pp. 652. ISBN 0-12-169160-8. \$82, £63.50.*

Ancient Australia. The Comprehensive Revision of Laserson's Acclaimed Work. Revised by RUDOLPH BRUNNSCHWEILER. *Angus & Robertson: 1984. Pp. 342. ISBN 0-207-14181-9. £20.*

The Apocalypses. Cancer and the Big Lie: How Environmental Politics Controls What We Know About Cancer. By EDITH EFRON. *Simon & Schuster: 1984. Pp. 589. ISBN 0-671-41743-6. \$19.95.*

The Arms Race at a Time of Decision: Annals of Pugwash 1983. JOSEPH ROTBLAT and ALESSANDRO PASCOLINI (eds). *Macmillan, London: 1984. Pp. 291. Hbk ISBN 0-333-37648-X; pbk ISBN 0-333-37649-8. Hbk £20; pbk £7.95.*

Artificial Intelligence: Human Effects. N. YAZDANI and A. NARAYANAN (eds). *Halsted: 1984. Pp. 318. ISBN 0-470-20092-8. £22.50.*

Atlas of Deep-Sky Splendors, 4th Edn. By HANS VEHREBERG. *Cambridge University Press: 1984. Pp. 242. ISBN 0-521-25834-0. £25, \$44.50.*

Australia and Nuclear War. MICHAEL DENBOROUGH (ed.). *Croom Helm: 1984. Pp. 270. Hbk ISBN 0-949614-05-X; pbk ISBN 0-949614-09-2. Hbk £19.95; pbk £8.95.*

The Biology and Evolution of Language. By PHILIP LIEBERMAN. *Harvard University Press: 1984. Pp. 379. ISBN 0-674-07412-2. £22.*

Bonsai: The Art and Technique. By DOROTHY S. YOUNG. Prentice-Hall: 1985. Pp.423. ISBN 0-13-079740-5. Np.

The Book of Edible Nuts. By FREDERIC ROSENGARTEN Jr. Walker: 1984. Pp.384. ISBN 0-8027-0769-6. \$35.

Bridge to the Future: A Centennial Celebration of the Brooklyn Bridge. MARGARET LATIMER, BROOKE HINDLE and MELVIN KRANZBERG (eds). New York Academy of Sciences: 1984. Pp.355. Hbk ISBN 0-89766-246-6; pbk ISBN 0-89766-247-4. Pbk \$75.

Circles in a Forest. By DALENE MATTHEE. Viking: 1984. Pp.368. ISBN 0-670-80009-0. £8.95.

The Cold and the Dark: The World After Nuclear War. By PAUL R. EHRLICH *et al.* (eds). Sidgwick and Jackson: 1984. Pp.229. ISBN 0-283-99146-1. £8.95.

Conceptual Issues in Alcoholism and Substance Abuse. J.H. LOWINSON and A. EINSTEIN (eds). Haworth Press: 1984. Pp. 102. ISBN 0-86658-316-4. \$19.95.

Current Topics in Chinese Science. Section A Physics, Section B Chemistry, Section 3 Mathematics, Section D Biology. Gordon & Breach: 1984. Section A Pp.490, ISBN 0-677-06220-6; Section B Pp.572, ISBN 0-677-06230-3; Section C Pp.769, ISBN 0-677-06240-9; Section D Pp.551, ISBN 0-677-06250-8. Np.

Determinants of Fertility in Developing Countries. Vol. 2 Fertility Regulation and Institutional Influences. RODOLFO A. BULATAO and RONALD D. LEE (eds). Academic: 1983. Pp.846. ISBN 0-12-140502-6. \$45.

Diamond. By GORDON DAVIES. Adam Hilger: 1984. Pp.255. ISBN 0-85274-512-5. £17.50.

Differential Inclusions. By J.P. AUBIN and A. CELLINA. Springer-Verlag: 1984. Pp.342. ISBN 3-540-13105-1/0-387-13105-1. DM 118, \$44.10.

Directory of Important World Honey Sources. By EVA CRANE, PENELOPE WALKER and ROSEMARY DAY. International Bee Research Association: 1984. Pp.384. Pbk ISBN 0-86098-141-X. Pbk £27.50.

Drug Design: Fact or Fantasy? G. JOLLES and K.R.H. WOOLRIDGE (eds). Academic: 1984. Pp.268. ISBN 0-12-388180-3. \$30, £18.

The Effects of Autism on the Family. ERIC SCHOPLER and GARY B. MESIBOV (eds). Plenum: 1984. Pp.363. ISBN 0-306-41533-X. Np.

Endangered Lives: Public Health in Victorian Britain. By ANTHONY S. WOHL. Methuen: 1983. Pp.440. ISBN 0-416-37950-8. Pbk £7.95.

Energy: Present and Future Options, Vol. 2. DAVID MERRICK (ed.). Wiley: 1984. Pp.394. ISBN 0-471-90416-3. £35.50.

Energy and Problems of a Technical Society. By JACK J. KRAUSHAAR and ROBERT A. RISTINEN. Wiley: 1984. Pp. 513. ISBN 0-471-86243-6. £25.50.

Environmental Diplomacy: An Examination and a Prospective of Canadian-U.S. Transboundary Environmental Relations. By JOHN E. CARROLL. The University of Michigan Press: 1983. Pp.382. ISBN 0-472-10029-7. \$22.50.

Environmental Warfare: A Technical, Legal and Policy Appraisal. ARTHUR H. WESTING (ed.). Taylor & Francis: 1984. Pp.107. Pbk ISBN 0-85066-278-8. Pbk £12, \$27.

Essays of an Information Scientist. By EUGENE GARFIELD. ISI Press: 1984. Pp.673. ISBN 0-89495-032-0. \$25.

Falconry in Arabia. By MARK ALLEN. Orbis: 1984. Pp.143. Pbk ISBN 0-85613-665-4. Pbk £7.99.

Federal Biotechnology Funding Sources. By OSKAR R. ZABORSKY and BRENDA K. YOUNG. OMEC: 1984. Pp.262. Pbk ISBN 0-931283-23-X. Pbk \$69.95.

Fellwalking with Wainwright: 18 of the Author's Favourite Walks in Lakeland. By A. WAINWRIGHT. Michael Joseph: 1984. Pp.224. ISBN 0-7181-2428-6. \$12.95.

The Fight for Food: Factors Limiting Agricultural Production. By HAROLD E. CROXALL and LIONEL P. SMITH. George Allen & Unwin: 1984. Pp.218. Hbk ISBN 0-04-6300112; pbk ISBN 0-04-630012-0. Hbk £15, \$25; pbk £4.95, \$7.95.

Food Analysis: Principles and Techniques. Vol. 1 Physical Characterization. DIETER W. GRUENWEDEL and JOHN R. WHITAKER (eds). Dekker: 1984. Pp.352. ISBN 0-8247-7181-8. SwFr. 166.

Food and Energy Resources. DAVID PIMENTEL and CARL W. HALL (eds). Academic: 1984. Pp.268. ISBN 0-12-556560-7. \$47.50, £33.50.

Food Protein Chemistry: An Introduction for Food Scientists. By JOE M. REGENSTEIN and CARRIE E. REGENSTEIN. Academic: 1984. Pp.353. ISBN 0-12-585820-5. \$52, £36.50.

Foundations of Supply-Side Economics: Theory and Evidence. By VICTOR A. CANTO, DOUGLAS H. JOINES and ARTHUR B. LAFFER. Academic: 1983. Pp.283. ISBN 0-12-158820-3. \$37.50.

The Gemini Syndrome. By R.B. CULVER and P.A. IANNA. Prometheus: 1984. Pp.222. Pbk ISBN 0-87975-264-5. Np.

George Parker Bidder: The Calculating Boy. By E.F. CLARK. KSL Publications, Knotting Green, Bedford MK44 1AA, UK: 1984. Pp.518. ISBN 0-9508593-01. £21.

Goldbach Conjecture. WANG YUAN (ed.). World Scientific: 1984. Pp.311. Hbk ISBN 9971-966-08-5; pbk ISBN 9971-966-09-3. Np.

Gums and Stabilisers for the Food Industry 2. Applications of Hydrocolloids. G.O. PHILLIPS, D.J. WEDLOCK and P.A. WILLIAMS (eds). Pergamon: 1984. Pp.569. ISBN 0-08-029819-2. £59.50.

Harpoon at a Venture: The Story of an Idyll that Went Sour. By GAVIN MAXWELL. Penguin: 1984. Pp.220. Pbk ISBN 0-14-006987-9. Pbk £2.95.

Hazardous and Toxic Materials: Safe Handling and Disposal. By HOWARD H. FAWCETT. Wiley: 1984. Pp.296. ISBN 0-471-80483-5. £35.80.

Heat Stroke and Temperature Regulation. M. KHOGALI and J.R.S. HALES (eds). Academic: 1984. Pp.312. ISBN 0-12-406180-X. \$36.50, £26.

The Hidden Universe. By MICHAEL DISNEY. J.M. Dent: 1984. Pp.216. ISBN 0-460-04441-9. £10.95

Highland Drove. By JOHN KEAY. John Murray: 1984. Pp.202. ISBN 0-7195-4105-0. £9.95.

The Idea of Economic Complexity. By DAVID WARSH. Viking: 1984. Pp.222. ISBN 0-670-23533-4. \$16.95.

Incomplete Data in Sample Surveys. Vol. 2. Theory and Bibliographies. WILLIAM G. MADOW, INGRAM OLKIN and DONALD B. RUBIN (eds). Academic: 1983. Pp.579. ISBN 0-12-363902-6. \$50.

Incomplete Data in Sample Surveys. Vol. 3. Proceedings of the Symposium. W.G. MADOW and I. OLKIN (eds). Academic: 1983. Pp.413. ISBN 0-12-363903-4. \$50.

Indirect Imaging. J.A. ROBERTS (ed.). Cambridge University Press: 1984. Pp.442. ISBN 0-521-26282-8. £27.50, \$54.50.

Inside Plea Bargaining: The Language of Negotiation. By DOUGLAS W. MAYNARD. Plenum: 1984. Pp.257. ISBN 0-306-41577-1. £29.50.

Instrumental Analysis of Foods: Recent Progress, Vol. 2. GEORGE CHARALAMBOUS and GEORGE INGLETT (eds). Academic: 1984. Pp.539. ISBN 0-12-168902-6. \$51, £39.50.

Iron Nutrition in Infancy and Childhood. ABRAHAM STEKEL (ed.). Raven: 1984. Pp.218. ISBN 0-89004-435-X. \$38.50.

Kenneth Clark: A Biography. By MERYLE SECREST. Weidenfeld & Nicolson: 1984. Pp.310. ISBN 0-297-78398-X. £12.95.

Last Days in Eden. By ELSPEETH HUXLEY and HUGO VAN LAWICK. Havrill: 1984. Pp.192. ISBN 0-00-272447-2. £9.95.

Life Histories and Psychobiography: Explorations in Theory and Method. By WILLIAM MCKINLEY RUNYAN. Oxford University Press: 1984. Pp.288. Pbk ISBN 0-19-503486-4. Pbk £9.50.

LIScraft. By ROBERT WILENSKY. W.W. Norton: 1984. Pp.384. Pbk ISBN 0-393-95442-0. Pbk £14.95.

Living and Working in Space: A History of Skylab. By W. DAVID COMPTON and CHARLES D. BENSON. NASA: 1983. Pp.449. \$20.

Lords of the Arctic: A Journey Among the Polar Bears. By RICHARD C. DAVIDS. Sidgwick & Jackson: 1984. Pp.140. Pbk ISBN 0-283-98971-8. £7.95.

Marihuana in Science and Medicine. By GABRIEL G. NAHAS. Raven: 1984. Pp.324. ISBN 0-88167-014-6. \$37.

The Marvel of Light: An Excursus. By ALFRED SCHMID. East-West Publications: 1984. Pp.299. ISBN 0-85692-110-6. £9.95.

Mathematical Byways in Ayling, Beeling and Ceiling. By HUGH APSIMON. Oxford University Press: 1984. Pp.97. ISBN 0-19-853201-6. £5.95.

The Messages of Tourist Art: An African Semiotic System in Comparative Perspective. By BENNETTA JULES-ROSETTE. Plenum: 1984. Pp.266. ISBN 0-306-41598-4. \$32.50.

The Mind and the Machine: Philosophical Aspects of Artificial Intelligence. STEVE TORRANCE (ed.). Halsted: 1984. Pp.213. ISBN 0-470-20104-5. £16.50.

Modeling Water Demands. J. KINDLER and C.S. RUSSELL (eds). Academic: 1984. Pp.248. ISBN 0-12-407380-8. \$28, £16.

Modern Falconry. By JACK SAMSON. Stackpole Books, PO Box 1831, Cameron & Kelker Streets, Harrisburg, Pennsylvania 17105, USA: 1984. Pp.160. Pbk ISBN 0-8117-2158-2. Pbk \$12.95.

Molten Salt Techniques, Vol. 2. ROBERT J. GALE and DAVID G. LOVERING (eds). Plenum: 1984. Pp.257. ISBN 0-306-41549-6. \$39.50.

Modern Techniques in Metrology. PAUL L. HEWITT (ed.). World Scientific: 1984. Pp.347. Hbk ISBN 9971-966-45-X; pbk ISBN 9971-966-46-8. Np.

New Directions in Helping, Vol. 3. Applied Perspectives on Help-Seeking and -Receiving. ARIE NADLER, JEFFREY D. FISHER and BELLA M. DE PAULO (eds). Academic: 1983. Pp.373. ISBN 0-12-257303-X. \$38.

Nicotine and the Tobacco Smoking Habit. D.J.K. BALFOUR (ed.). Pergamon: 1984. Pp.221. ISBN 0-08-030779-5. £40, \$65.

On a New Method of Analysis and Its Applications. By PAUL TURÁN. Wiley: 1984. Pp.584. ISBN 0-471-89255-6. Np.

One Man's Island: A Naturalists' Year. By KEITH BROCKIE. Harper & Row: 1984. Pp.150. ISBN 0-06-015360-1. \$19.95.

Only When I Eat (Tinnitus, Hope at Last). By JUNE ROGERS. Regency Press, 125 High Holborn, London WC1V 6QA: 1984. Pp.32. Pbk £1.50.

The Origins of Man and the Universe: The Myth that Came to Life. By BARRY LONG. Routledge & Kegan Paul: 1984. Pp.300. Pbk ISBN 0-7102-0337-3. Pbk £6.95.

Oxygen and the Conversion of Future Feedstocks. THIRD BOC PRIESTLEY CONFERENCE. Royal Society of Chemistry: 1984. Pp.471. Pbk ISBN 0-85186-915-7. Pbk £18, \$33.

Petroleum: How it is Found. By WILLIAM R. PAMPE. Enslow: 1984. Pp.96. ISBN 0-89490-100-1. \$9.95.

Physical Structure of Olympic Athletes. Medicine and Sport Science 18. J.E.L. CARTER (ed.). Karger: 1984. Pp.248. ISBN 3-8055-3871-5. DM 188, \$94.

Pocket Book of Dinosaurs. By MICHAEL BENTON. Kingfisher: 1984. Pp.188. ISBN 0-86272-122-1. £3.95.

Profitability of Food Processing. By INSTITUTION OF CHEMICAL ENGINEERS. Pergamon: 1984. Pp.430. ISBN 0-08-030279-3. £38, \$61.

Programming Effective Human Services: Strategies for Institutional Change and Client Transition. W.P. CHRISTIAN, G.T. HANNAH and T.J. GLAHN (eds). Plenum: 1984. Pp.514. ISBN 0-306-41526-7. \$45.

Progress in Combinatorial Optimization. WILLIAM R. PULLEYBLANK (ed.). Academic: 1984. Pp.374. ISBN 0-12-566780-9. \$43.50, £30.50.

Protection of Human Research Subjects: A Practical Guide to Federal Laws and Regulations. By DENNIS M. MALONEY. Plenum: 1984. Pp.420. ISBN 0-306-41522-4. Np.

The Pyramid and the Grail: Towards a Parallel Reality. By MICHAEL BECKETT. Lallouen Press, 5 Hayter Gardens, Romsey, Hants SO5 8QU, UK: 1984. Pp.124. Pbk ISBN 0-95095590-6. Pbk £4.95.

Reagan and the World: Imperial Policy in the New Cold War. By JEFF MCMAHAN. Pluto: 1984. Pp.214. Pbk ISBN 0-86104-602-1. Pbk £3.95.

Recent Advances in Phytochemistry, Vol. 18. Phytochemical Adaptations to Stress. BARBARA N. TIMMERMAN, CORNELIUS STEELINK and FRANK A. LOEWUS (eds). Plenum: 1984. Pp.334. ISBN 0-306-41720-0. \$49.50.

Rule-Based Expert Systems: The MYCIN Experiments of the Stanford Heuristic Programming Project. By BRUCE G. BUCHANAN and EDWARD H. SHORTLIFFE. Addison-Wesley: 1984. Pp.748. ISBN 0-201-10172-6. Np.

Blanket, mask and cream

New this month are several items aimed at lab safety—including a fire-fighting blanket, a protective face mask and protective skin cream.

● For molecular cloning, Bio-Rad has introduced a T4 DNA ligase that can be used both to join double-stranded DNA molecules in recombinant applications and to repair single-stranded nicks in duplex DNA. Capable of ligating either cohesive-end or blunt-end DNA, the T4 DNA ligase is purified from an *Escherichia coli* strain and qualified for molecular cloning experiments by a number of quality assurance procedures. These include: monitoring ligation efficiency on both cohesive and blunt-end fragments; an endonuclease and an exonuclease activity assay and a ligation/recutting assay.

Reader Service No. 100.

● Celltech's range of reagents for the purification and assay of interferons now includes Beta Sepharose, a reagent based on monoclonal antibodies and intended for the purification of interferon beta. Beta Sepharose gives a single-step chromatographic process that can virtually eliminate all impurities and retain a high yield of active interferon beta.

Reader Service No. 101.

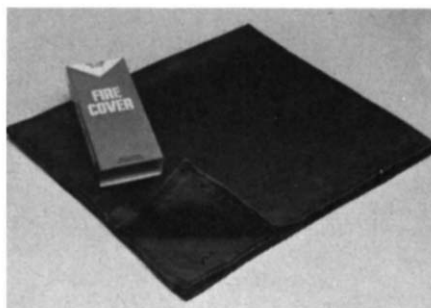
● A free newsletter available from Lab Safety Supply of Jamesville, Wisconsin, consists of abstracts of significant publications in the area of chemical health and safety. Subjects include personal protection, laboratory safety, safe handling, storage and disposal of toxic and hazardous chemicals, biosafety and radiation safety. For a complimentary copy of "Chemical Health & Safety Abstracts" use the Reader Service Card.

Reader Service No. 102.

● Three leading producers of dyes and stains for microscopy have collaborated to produce a range which microscopists can rely upon to give accurate results from batch to batch. Brand-named Certistain, the new range results from an agreement between BDH Chemicals Ltd, EM Diagnostic Systems Inc., and E. Merck, on individual stain specifications covering both chemical criteria and practical biological testing. The Certistain range initially contains 30 stains and this will be expanded over the next few years to a total of around 200. Supplies of Certistain dyes and stains can be obtained from any of the three companies, their associates, agents or distributors throughout the world. A comprehensive handbook on the new Certistain range can be obtained through the Reader Service Card.

Reader Service No. 103.

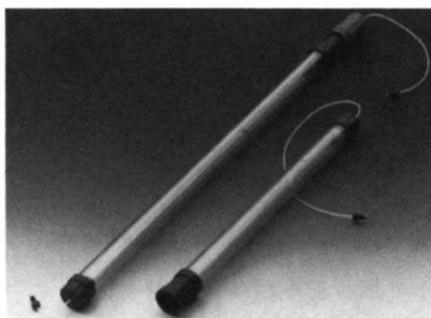
These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



The graphite-impregnated Fire Cover.

● The Fire Cover fire blanket, a completely new fire fighting aid made of fibreglass cloth impregnated with graphite and Teflon, is now available from Bel-Art Products. The blanket is capable of extinguishing small laboratory and fume hood fires, as well as Class A, light oil and grease fires. The 39" x 43" Fire Cover fire blanket is packaged in a small instant-access container, which can be hung on walls or stored in any convenient place. The graphite impregnated in the cover rapidly absorbs heat generated by burning material and helps lower the flashpoint below a substance's re-ignition temperature. The close weave of the cover prevents atmospheric oxygen from getting into and feeding the burning materials.

Reader Service No. 104.



Pharmacia's new FPLC columns.

● Column HR 16/50, a new high-resolution chromatography column, and Packing Equipment HR 16, for efficient column packing, are specially designed for high performance preparative separations with the Pharmacia fast protein liquid chromatography (FPLC) system. The HR 16/50 column (for bed dimensions of 16 x 500mm) is particularly well suited for chromatography of biomolecules using the Pharmacia gels Superose 6B, Sepharose CL-6B and Sephacryl S-types. The HR 16/50 column and the Packing Equipment HR 16 are fully biocompatible and can be used at pressures up to 30 bar (3 MPa). The pressure specification allows efficient packing at higher flow rates.

Reader Service No. 105.

● Bio-Yeda now offers conjugated affinity purified goat antibodies directed against mouse Fab and mouse Fc fragments. These antibodies are particularly suited as secondary reagents in work with mouse monoclonal antibodies. They may be used in hybridoma screening with the ELISA technique and as instruments for detection of monoclonal antibodies on tissue or cell preparations using indirect immunoenzymatic or immunofluorescence methods. These affinity purified anti-mouse antibodies are exhaustively adsorbed with human serum proteins to assure minimal cross-reaction with human proteins on tissue or cell preparations. The anti-mouse Fab products offer sensitive and broad activity for all mouse immunoglobulins (IgG, IgA, IgM, IgE) where the anti-mouse Fc products react specifically with mouse IgG (all subclasses). Affinity purified antibodies to mouse Fab and mouse Fc are available unmodified or as fluorescein, peroxidase and alkaline phosphatase conjugates.

Reader Service No. 106.



The LKB Novaspec for visible spectroscopy.

● For applications where a highly sophisticated spectrophotometer is unnecessary, LKB has introduced the Novaspec, a simple inexpensive visible spectrophotometer made to modern optical standards. Simplicity of operation is ensured with only four keys controlling the instrument's operation. The Novaspec features automatic wavelength calibration at switch-on, and microprocessor controlled selection of correct filter combinations. The wavelength range is broad, and a universal cell holder adds to the instrument's versatility. For applications in the teaching laboratory, the LKB Novaspec is fully complemented by a range of educational accessories, including a textbook on the basic principles of spectrophotometry, and a wall chart summarizing these principles in a graphic way.

Reader Service No. 107.

● Tecator has developed a fully automated flow injection analysis system to bring the techniques to a larger number of applications than previously possible. FIAstar is intended primarily for the fast, simple analysis of water, soil, fertilizers and beverages. This use of the FIA technique also opens up the possibility of new applications in wet chemistry analyses. *Reader Service No. 108.*

● Recent additions to the Sera-Lab range include four new monoclonal antibodies against mouse T-cells for use in *in vitro* and *in vivo* depletion of T-cells and T-cell subsets. Applications include the investigation of the role of different T-cells in models for infectious disease, autoimmunity, tolerance, neoplasia and transplantation, and also in cell sorting. *Reader Service No. 109.*

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The Gonotec automatic osmometer.

● A fully automatic osmometer using the depression of freezing point method for measurements of total osmolality in under one minute, is now available from Clandon Scientific Limited. The Gonotec Model 030 osmometer requires only 50 µl of sample (or 30 µl with lower precision) and displays results in osmol per kg. The instrument is very simple to use, being fully automatic after sample presentation. Measuring 210 × 360 × 240 mm, the 030 weighs approximately 9kg and can be connected to a recorder or printer.

Reader Service No. 110.



Locount cream protects the cleanroom environment from skin particles, and the skin from irritation.

● A new skin cream has been developed especially to solve problems with skin particulation and skin irritation in research and cleanroom environments. LoCount, from VWR Scientific absorbs right into the skin and has no particles in suspension to dry skin as oil-based creams do. The cream reduces skin particulation by as much as 90%. Its completely organic formulation means that standard cleaners easily take LoCount off any surface. LoCount is low in ionics and halogens, so if someone accidentally contaminates a device with the cream, LoCount presents minimal risk to the performance of an electrical circuit. Use of the cream reduces skin discomfort from wearing vinyl gloves for long periods, and the cream is hypoallergenic.

Reader Service No. 111.

Availability of frozen serum panels

A VARIETY of serum components have been reported to be useful in cancer diagnosis and/or in monitoring cancer treatment or recurrence. The US National Cancer Institute is interested in evaluating serum assays potentially useful in cancer diagnosis. Coded panels composed of 1 ml aliquots of pretreatment frozen sera from patients with various neoplasms, from benign disease patients, and from healthy controls are available to investigators to evaluate assays in which preliminary results indicate the ability to discriminate between cancer patients and controls. Promising results may form the basis for a subsequent grant application. Preliminary data documenting a useful test must be submitted and should include: a brief description of the assay, results in patients with cancer and in non-malignant disease, results in healthy controls and reprints of published work if available. Requests should be sent to: Diagnosis Serum Panels, Project Officer NCI-Serum Bank, Room 10A10, 5333 Westbard Avenue, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205.

Reader Service No. 112.

● A new range of calibrated lattices has been developed by Rhône-Poulenc. These Estapor lattices make it easier to detect the complex formed by the immunological reaction between antigen and specific antibody. Calibrated lattices, because of their high uniformity, are increasingly used in diagnostic tests such as agglutination, ELISA, RIA and erythrocyte replacement. Other applications include electron microscopy, filtration and chromatography. Estapor lattices are supplied with or without functional group and are available in either coloured, fluorescent or magnetic forms.

Reader Service No. 113.

● The Laboratory Facelet, produced by Charcoal Cloth Ltd, offers a high level of protection against toxic fumes, vapours, gases, odours and particles. The Laboratory Facelet is a derivative of the military facelet approved to NATO standards and currently in supply to the British armed forces for initial protection against chemical attack. Like the military version, the Laboratory Facelet incorporates activated Charcoal Cloth — a material composed entirely of 100% charcoal fibres which has a far higher rate of adsorption for gases and vapours than granular charcoal or charcoal-impregnated materials. In addition, other filtration materials have been included to provide a high level of protection against a particulate threat. The facelet features a low breathing resistance, a high level of speech transmission, and is light and comfortable. The mask is re-useable and much cheaper than conventional respirators.

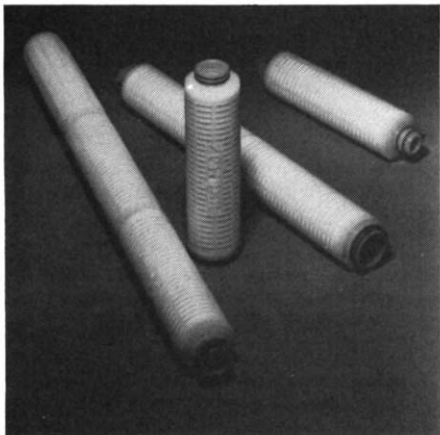
Reader Service No. 114.



A new digital thermometer from Automatic Systems.

● Automatic Systems Laboratories, of Milton Keynes, has introduced a new digital thermometer which is capable of absolute and differential temperature measurements with a resolution of 0.001°C between minus 270°C and plus 630°C . The digital readout, even at high resolution, is completely stable. The new micro-processor-controlled instrument gets its extreme stability from the use of low frequency a.c. techniques, which eliminate the thermally induced voltages and drifts associated with d.c. systems.

Reader Service No. 115.



AMF Cuno's new polypropylene pleated filter.

● The AMF Cuno Polypro is a high-surface-area, all-polypropylene pleated filter designed for critical filtration applications. Polypro cartridges are produced by pleating multiple layers of a non-fibre releasing polypropylene matrix. The 100% polypropylene construction assures compatibility with a wide range of solvents. Five absolute micrometre ratings are available from 0.6 to $10.0\ \mu\text{m}$. Applications include pharmaceutical solvents, high-purity water and photographic emulsions.

Reader Service No. 116.

● Available from Medical Systems Corporation of Green Neck, New York, The Model MF-83 Microforge is specifically designed by the Narishige Instrument Laboratory of Japan for polishing glass micropipettes used for patch clamping. The MF-83 has a magnification of $600\times$, and the $40\times$ objective features a long working distance. Incorporated in the Microforge are: an adjustable lamp and heat control; an external foot switch, which controls the heating of the glass micropipettes; full manipulation of both the glass pipette and the heating element.

Reader Service No. 117.

Government Chemist opens the doors

THE Laboratory of the Government Chemist, under the auspices of the UK Department of Trade and Industry, is now offering its range of materials testing facilities for contract use by the private sector. The LGC has been actively involved with materials research and development for over 20 years. Work began in the field of dentistry but has now moved into other industrial areas such as coatings technology and controlled-release. Among the facilities available at LGC are techniques for measuring:

- Mechanical properties — including strength, hardness, roughness, abrasion resistance and fatigue.
- Rheological properties.
- Chemical and microstructural analysis.
- Colour measurement and translucency.
- Thermal properties.
- Environmental weathering properties.

These facilities can be used on a regular or ad hoc basis for investigating the properties of polymers, ceramics, glasses, metals, coatings, rubbers, cements and dental materials and also for solving problems associated with materials in industry.

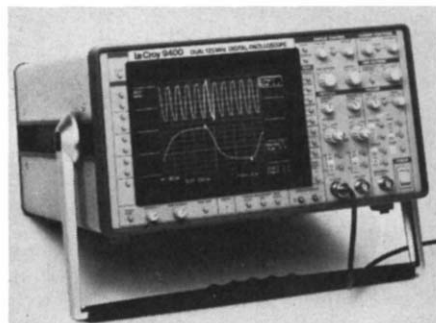
Reader Service No. 118.

● A comprehensive range of monoclonal antibodies for tissue typing is now available from Amersham International. Seven monoclonals make up Amersham's range for identifying a cell's intermediate filament protein structure. The new products are anti-cytokeratin, anti-desmin, anti-vimentin, three anti-neurofilaments and anti-gial fibrillary acidic protein. For those wishing to classify unknown tissue, Amersham offers a composite pack of basic antibodies necessary for tissue typing, while for those studying particular types of cell all the products are available separately. In addition to this new intermediate filament range, Amersham already has an established line of monoclonal antibodies for typing other intracellular structures.

Reader Service No. 119.

● The Metachem Diagnostics Cell-ect is a cost-effective system for separating cell populations without the need for expensive instrumentation. Cell-ect kits provide the reagents and instructions necessary to perform 10 separations. The principle of the system is to prepare polystyrene dishes with a specific ligand. A cell suspension is poured onto the plate and incubated. The non-adherent cells are removed by decanting the supernatant, while the adherent cells are recovered by pipetting. The kits currently available are: total histamine (TH), for the separation of all histamine receptor bearing cells; histamine receptor 2 (H_2), for the separation of Type 2 receptor bearing (H_2R^+) cells; and human Ig, murine Ig, for the separation of Ig-bearing cells from non-Ig bearing cells.

Reader Service No. 120.



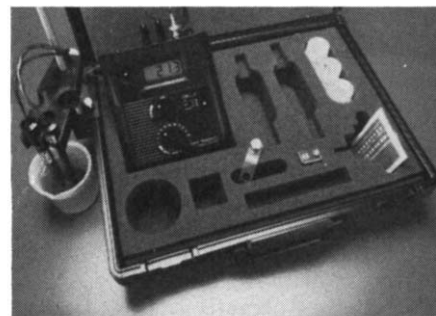
The Lecroy 9400 combines an oscilloscope with the function of a transient recorder.

● The Lecroy 9400 Dual 125-MHz digital storage oscilloscope combines the advantages of an oscilloscope and a transient recorder in an easy-to-use and portable (14-kg) instrument. The 9400 features complete programmability and extensive interfacing options for remote control and computer archiving, with a wide 125-MHz analog bandwidth. Both input channels are identical, with accurate 100 megasample per second, 8-bit analog-to-digital converters and very large 32K work acquisition memories.

Reader Service No. 121.

● To facilitate studies in the field of oncogene research, Triton Biosciences Inc. is now marketing affinity-purified antibody to Ha-ras p21 protein. Antisera are generated in sheep against a variety of synthetic peptides corresponding to potential antigenic sites on the native protein molecule. Antibodies are purified by peptide affinity column chromatography and are characterized by Western blot and immunoprecipitation analyses.

Reader Service No. 122.



Orion's portable meter.

● Orion Research Incorporated has introduced two new meters for pH, DO/BOD, and redox potential measurements in the lab or field. The meters, SA 210 and SA 230, have measuring ranges of $0-14\ \text{pH}$ and $+1,999\ \text{mV}$. The SA 210 is a pH/mV meter. The SA 230 is a pH/temperature/mV meter with ATC probe for automatic temperature compensation. Both meters come with a rugged carrying case, all the essentials needed for measuring pH, and a special electrode holder that attaches to the carrying case, allowing the meter to remain in the case during measurements. Battery life is approximately 100-150 hours, continuous use, and an a.c. line adapter is available.

Reader Service No. 123.

The Cambridge phenomenon

from Richard Pearson

As science parks continue to proliferate throughout the United Kingdom, it remains difficult to define the ingredients for success

HIGH technology and small businesses are seen as two essential routes to job creation in both Europe and North America. As a result, policy makers are looking for the key to unlock such job creation potential by stimulating the development and utilization of high technology, and by aiding and encouraging the would-be entrepreneur. For some, science parks are the magic catalyst which draws the two phenomena together. By observing the success of Silicon Valley in the United States, which itself was spawned by Stanford University via its science park, and now has 200,000 jobs, every higher education institution and local authority in the United Kingdom now has plans or ideas for developing its own version of a science park. For those thinking on a grander scale, then the replication of Silicon Valley itself, for example as in Silicon Glen in Scotland, which has developed as a result of public policy initiatives and now has 40,000 jobs, or the M4 sunbelt in England where growth has been more spontaneous, is the ideal translation from high technology to job creation. In the United Kingdom the concept of the science park is taking many different forms, ranging from, at one extreme, the re-labelling of a high quality industrial estate in pleasant surroundings, to the park located adjacent to and on land owned by a higher education institution. There are now over 40 such initiatives under way.

The Cambridge Science Park, formed in 1973, and owned and developed by the University of Cambridge, has been one of the UK pioneers. In addition, the area has also spawned the 'Cambridge phenomenon', the apparently spontaneous emergence of a cluster of high technology companies along the lines of Silicon Valley. As well as being the home for Sinclair and Acorn, two of the biggest microcomputer companies in the world, the area contains over 300 other high technology companies, employing nearly 14,000 people between them and accounting for one in six local jobs. Their total turnover in 1984 has been estimated at £890 million¹. While the first high technology company to come out of Cambridge University can be traced back to 1881, when Cambridge Scientific Instrument Company was formed by Charles Darwin's son to manufacture scientific equipment for the university, the Cambridge phenomenon can be said to have really taken off some 100 years later. Over the past 20 years one new company has been established in the area every month, with peak rates of establishment occurring in 1978, 1981 and 1983 when over 2 per month were being established (Fig. 1).

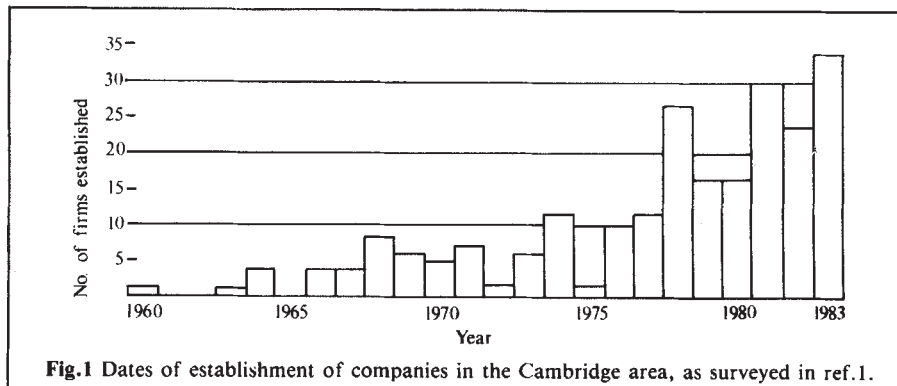


Fig. 1 Dates of establishment of companies in the Cambridge area, as surveyed in ref. 1.

Three quarters of these companies are new independent enterprises with the balance being made up of new branches of existing UK or overseas firms, or companies moving into the area. There is a high preponderance of very small companies in the total, one in three having five or fewer employees or an annual turnover of under £400,000. Electronics and computer hardware are the dominant business activities, but there is also a strong representation in biotechnology. A significant proportion of the companies' activities centre on research, design and development and consultancy.

The origins of the vast majority of these companies can be linked to the university, but only in a few cases, one in six, have they been formed by people coming straight from the university. For the majority it is because key staff have left one local company to start their own business in the area. This interconnection between companies, together with associated mobility of professional staff between companies, is seen as one of the great strengths and ingredients for success in both the formal settings of science parks, where the flows are expected to be between the educational establishment and industry, and in the 'Silicon Valleys', where it is between the companies involved. An additional asset of an area like Cambridge is the social network.

A distinguishing feature of high technology companies is the high qualification profiles of their workforces, a factor reinforced by the separation of manufacturing facilities to distant, and often off-shore locations. In the Cambridge case, one in three of the staff in the companies were either managers or professional scientists and engineers, with the most recently formed companies having over half their staff in these categories. While shortages of such people are operating as a constraint in many locations, Cambridge does not seem to be suffering too badly. In part this is a result of the large supply of professionally trained graduates being produced by the

university, but also the attractive location is an attraction to would-be migrants.

For many, the whole idea of a science park is that of the interplay of ideas between higher education institutions and local companies. In the Cambridge case, a study in 1983² showed that there were surprisingly few formal linkages between the companies on the park, and that companies located elsewhere in the area were just as likely to have research contracts, consultancies and teaching commitments with the university. Such linkages would seem to find more expression through informal social contacts, ease of recruitment, and a 'halo' effect which stimulates action and change by example, rather than simply as a result of close physical proximity.

What, then, are the implications for other locations seeking to encourage new high technology firms? The first is that Cambridge has many special features which are unlikely to be repeated in many other areas. The second is that such developments take time, the Cambridge phenomenon had its beginnings many decades ago, with the science park having taken over 10 years to come to full fruition, and the park itself is only one small part of the process. Finally, if US experience is anything to go by, for every success, there will be a series of still-born or struggling parks. Science parks and high technology 'clusters' may have emerged quickly in the fashion stakes; the rewards for the successful will, however, come over a much longer time span, and while initiatives by public agencies can aid this process, 'natural' advantages will be just as important, and many of these are still not well understood □

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1. *The Cambridge Phenomenon* (Seagal, Quince and Partners, Cambridge 1985).

2. Moore, B. & Spires, R. *The Experience of the Cambridge Science Park* (OECD, Paris, 1983).